Fig. 1

NORMAL CELL

Injury / Stress
Genetic Mutations
Cytokines
Growth Factors

Second Messengers

ERK Pathway
Restricted

ERK   JNK   P38

c-Fos

c-Jun

c-Fos
c-Jun

AP-1 Activation

AP-1 Dependent Genes

Cell Proliferation
Migration

Cytokines and
Inflammatory
Mediators

MMPs
Matrix
Metalloproteinases

Angiogenesis

Fig. 2

TRANSITIONAL STATE

NORMAL CELL

DISEASED CELL

IF SUSTAINED

Fig. 3

−Focal Adhesions (−FA)

Fig. 4A

+Focal Adhesions (+FA)
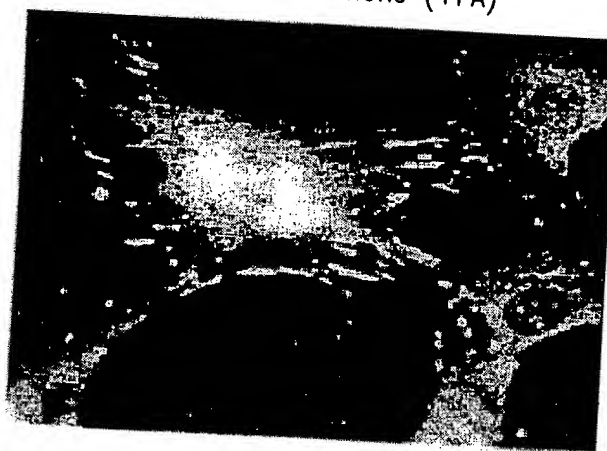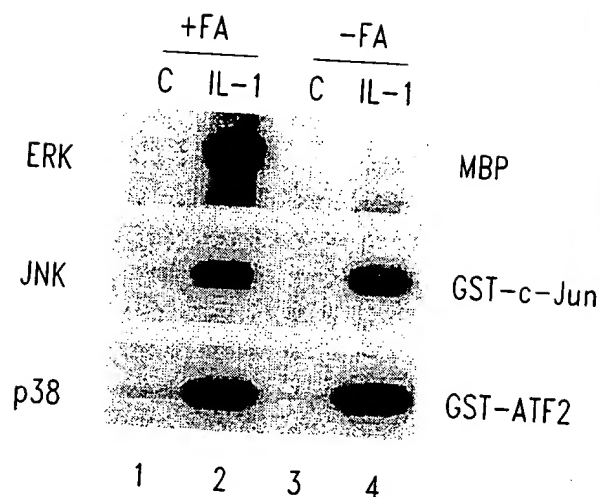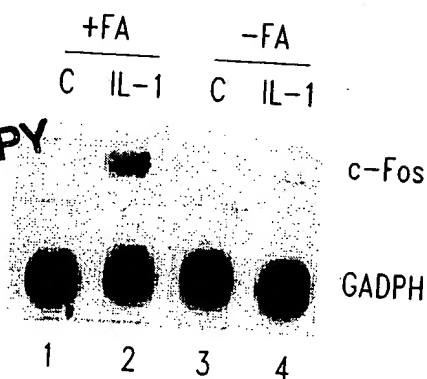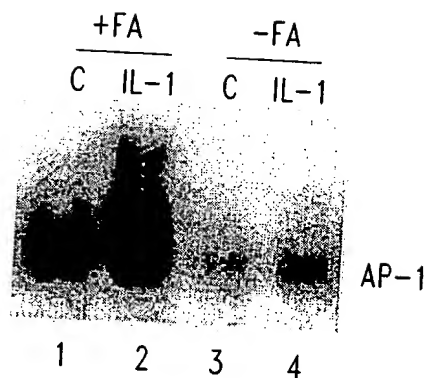
+FA   −FA
C   IL−1   C   IL−1

ERK   MBP

JNK   GST−c−Jun

p38   GST−ATF2

1   2   3   4

Fig. 4C

Fig. 4B

+FA   −FA
C   IL−1   C   IL−1

c−Fos

GADPH

1   2   3   4

Fig. 4D

+FA   −FA
C   IL−1   C   IL−1

AP−1

1   2   3   4

Fig. 4E

Fig. 5

10% FCS

ERK2 activity		MBP
phospho-MAPK		p44 ERK1 p42 ERK2
ERK2 level		ERK2

10T1/2   LR21

Fig. 6A

10T1/2
LR21

AP-1

Fig. 6B

Fig. 7A

Fig. 7B

PL   FN

10T1/2   LR21   10T1/2   LR21

c-fos

c-jun

GAPDH

Fig. 8

RHAMM   RHAMM

−   +   −   +

gelatinase B   TIMP-1

stromelysin   GAPDH

GAPDH

Fig. 9

Fig. 10

	−RHAMM			+RHAMM		
	0	30	60	0	30	60

c-fos

GAPDH

IL-1

Fig. 11A

	−RHAMM			+RHAMM		
	0	30	60	0	30	60	[min]

c-fos

GAPDH

TNFα
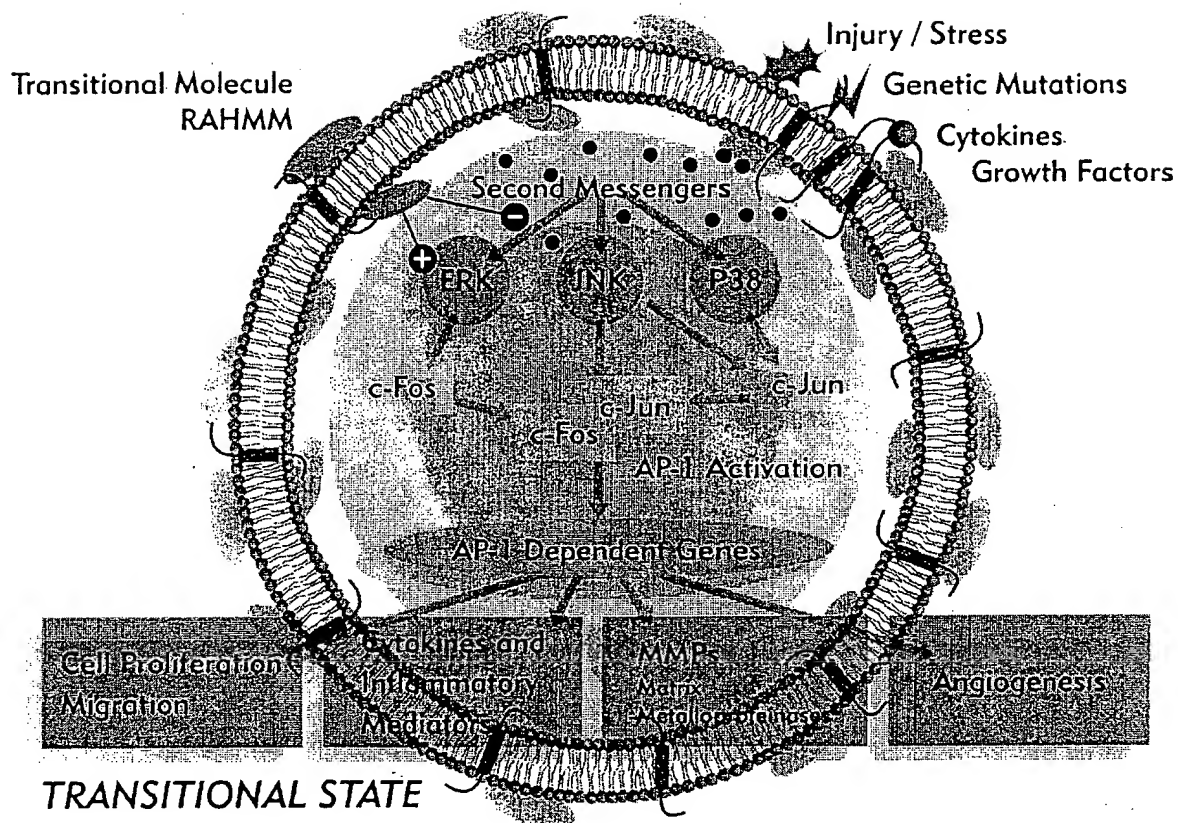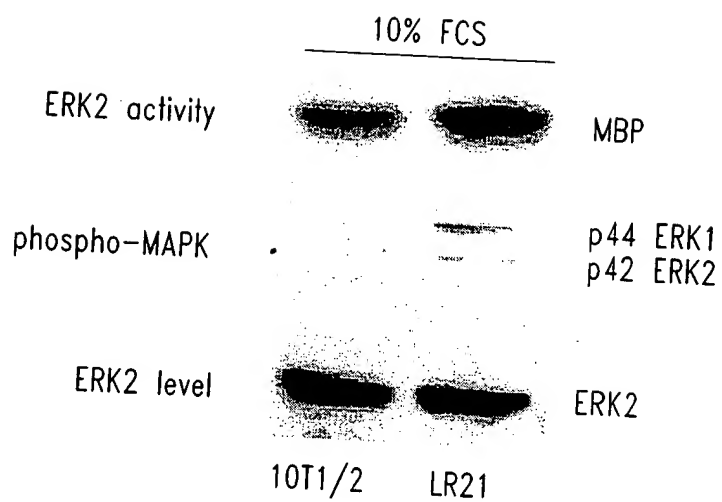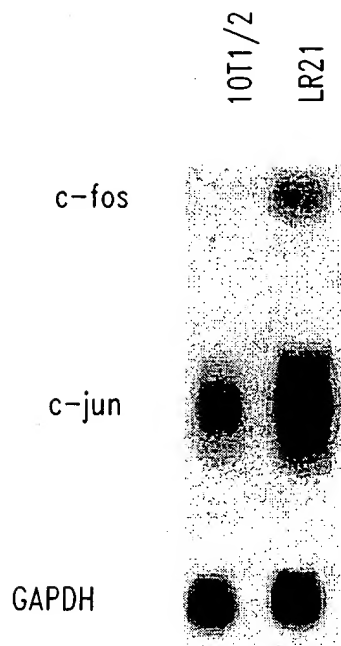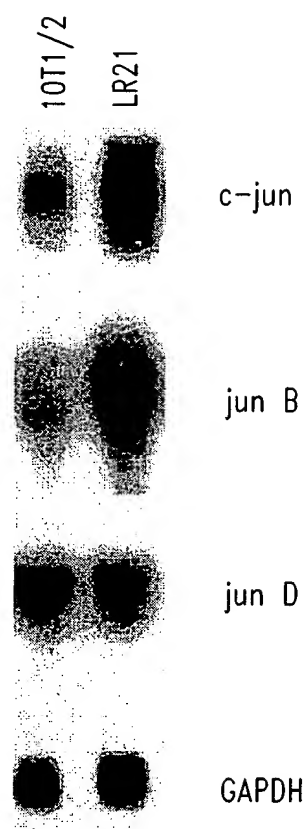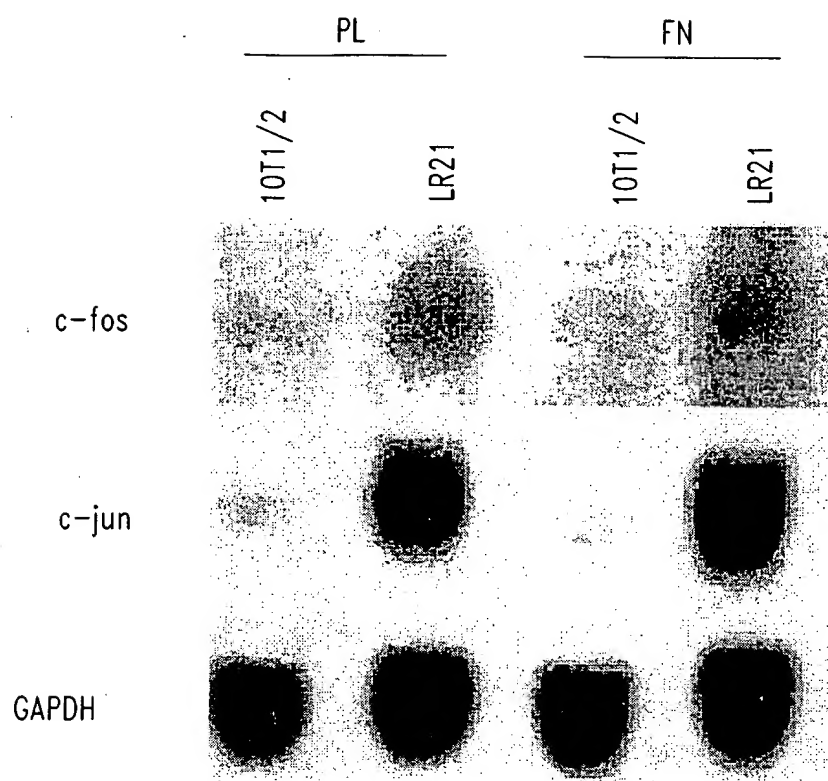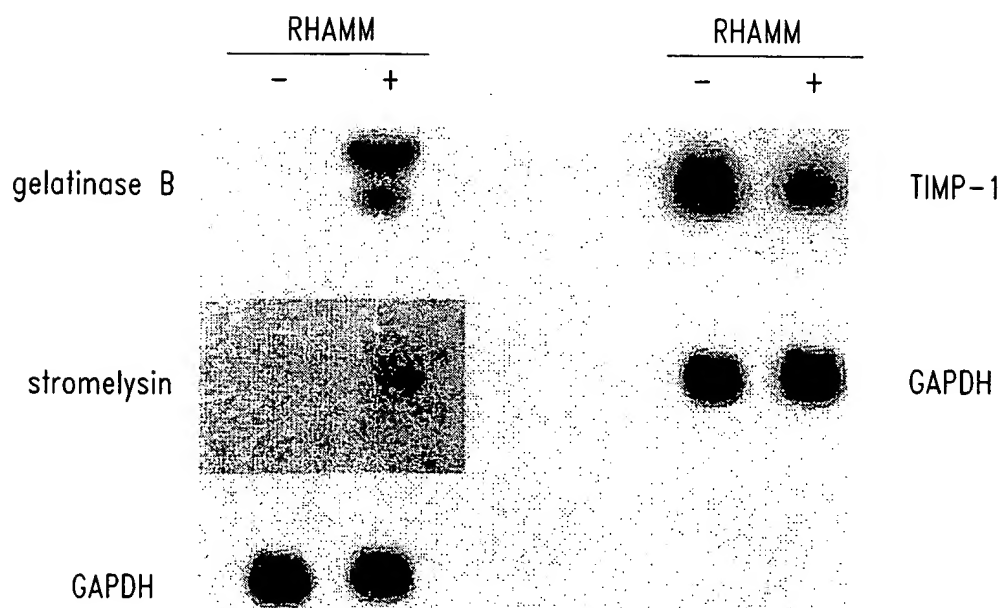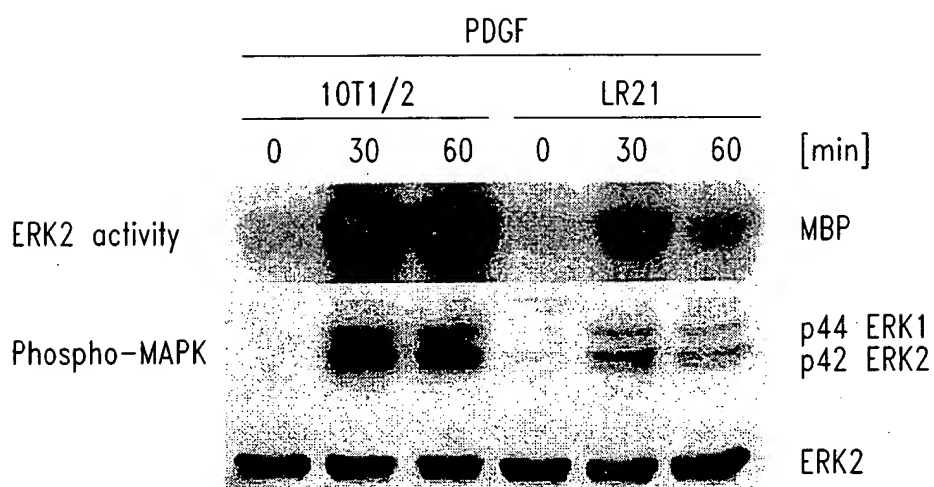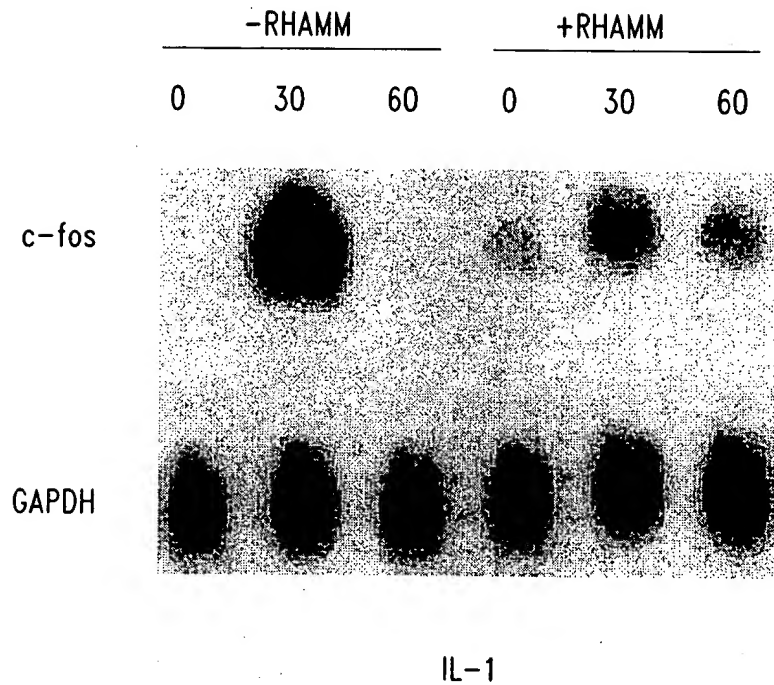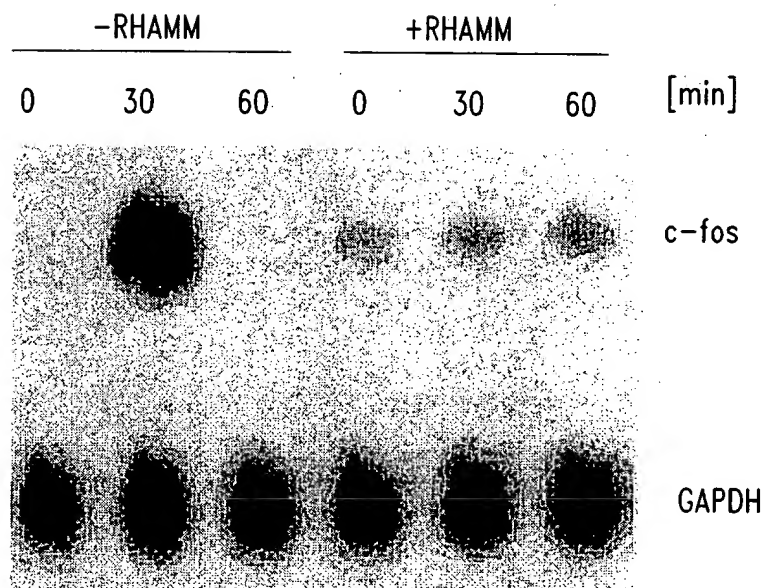
Fig. 11B
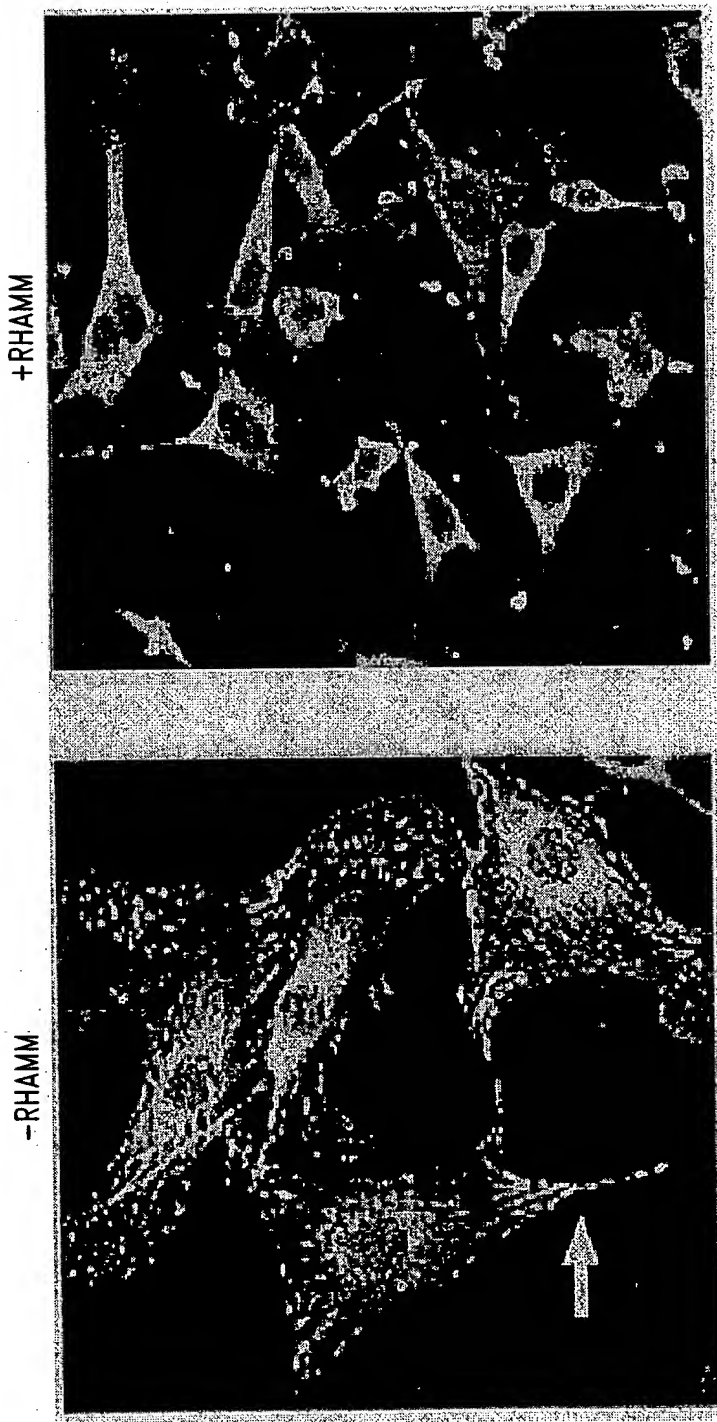
−RHAMM

+RHAMM

+RHAMM cells make less focal contacts than their parental cells (−RHAMM)

Fig. 12A

Fig. 12B

Fig. 13

- Overexpression of RHAMM results in increased and sustained production of podosomes
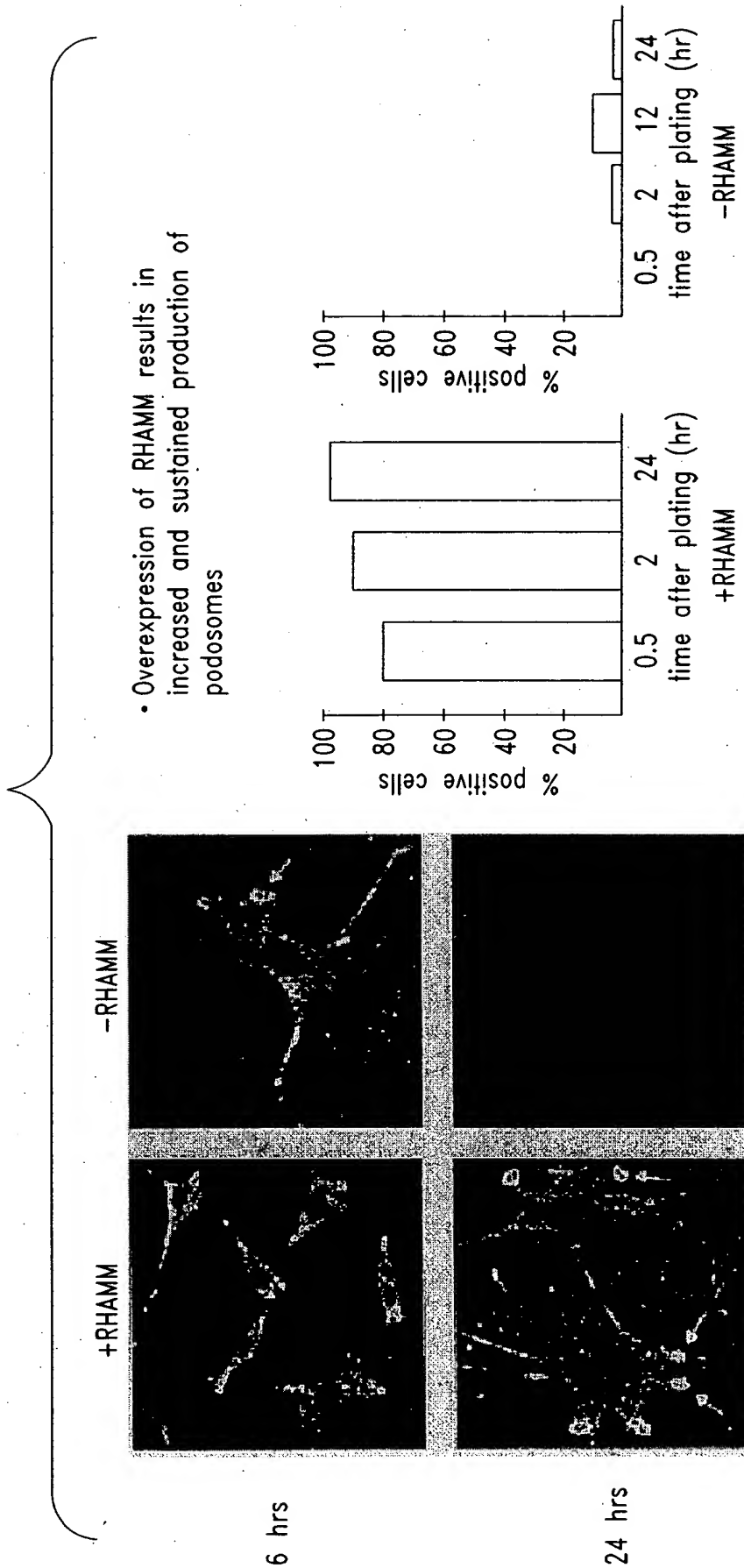
- In most cells responding to injury, podosomes are formed and disassembled rapidly (-RHAMM, 6 vs. 24 hrs)
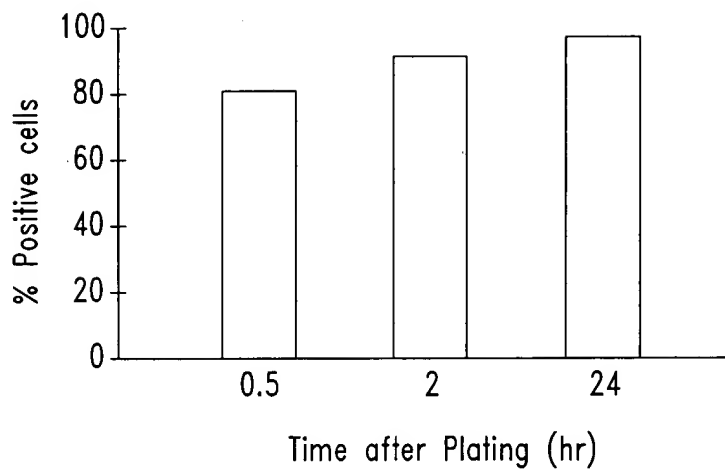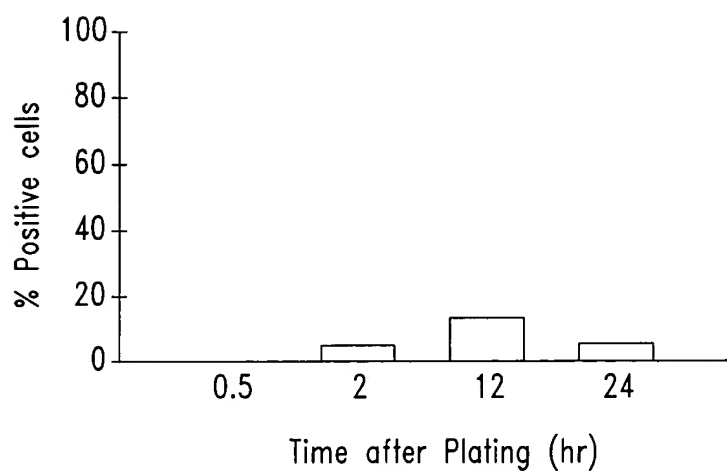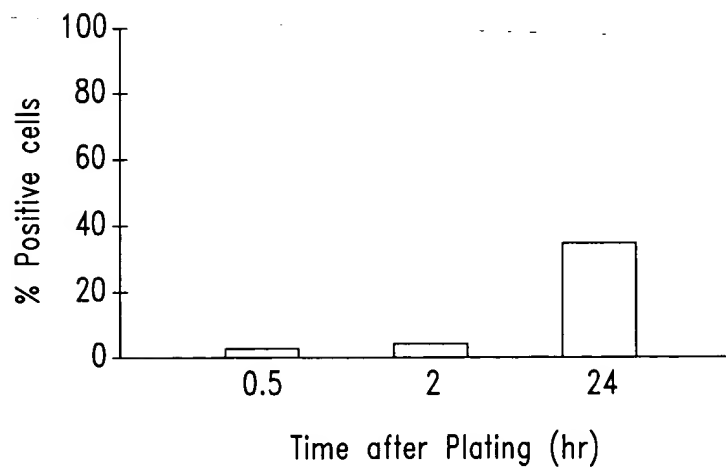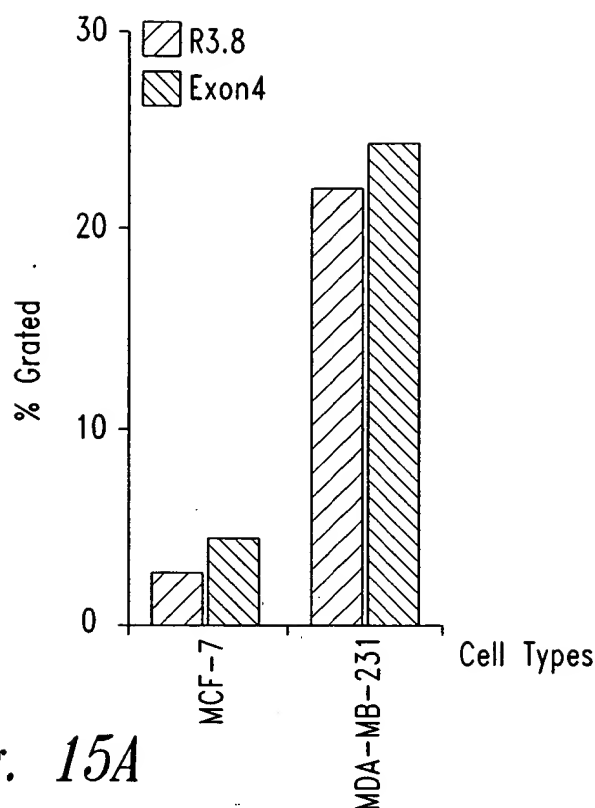
Fig. 14A

Fig. 14B

Fig. 14C

Fig. 15A

RHAMM Peptides

Murine Exon3 sequence:
      N-terminal ---KLQATQKDLTESKGKIVQLEGKL--- 23aa   SEQ ID. NO. 14

For Exon3 antibody, used the peptide sequence:
          (C) KLQATQKDLTESKG   SEQ ID. NO. 15

Murine Exon4 sequence:
      N-terminal ---VSIEKEKIDEKCETEKLLEYIQEIS--- 25aa   SEQ ID. NO. 16

For Exon4 antibody, used the peptide sequence:
          (C) VSIEKEKIDEKC/S   SEQ ID. NO. 17

For antibody to Human RHAMM v5, used the peptide sequence:
          (C) LKSKFSENGNQKNL   SEQ ID. NO. 18

Homology between three peptides from murine (M) and
human (H) RHAMM (as used to raise antibody)

1) Exon3	M:	KLQATQKDLTESKG	as in SEQ ID. NO. 15
	H:	---V--RS-E-Q--	SEQ ID. NO. 19
2) Exon4	M:	VSIEKEKIDEKC	as in SEQ ID. NO. 17
	H:	----------S	as in SEQ ID. NO. 17
3) v5	M:	--A----D-H---M	SEQ ID. NO. 20
	H:	LKSKFSENGNQKNL	as in SEQ ID. NO. 18

Fig. 15B

peptide 1

peptide 2
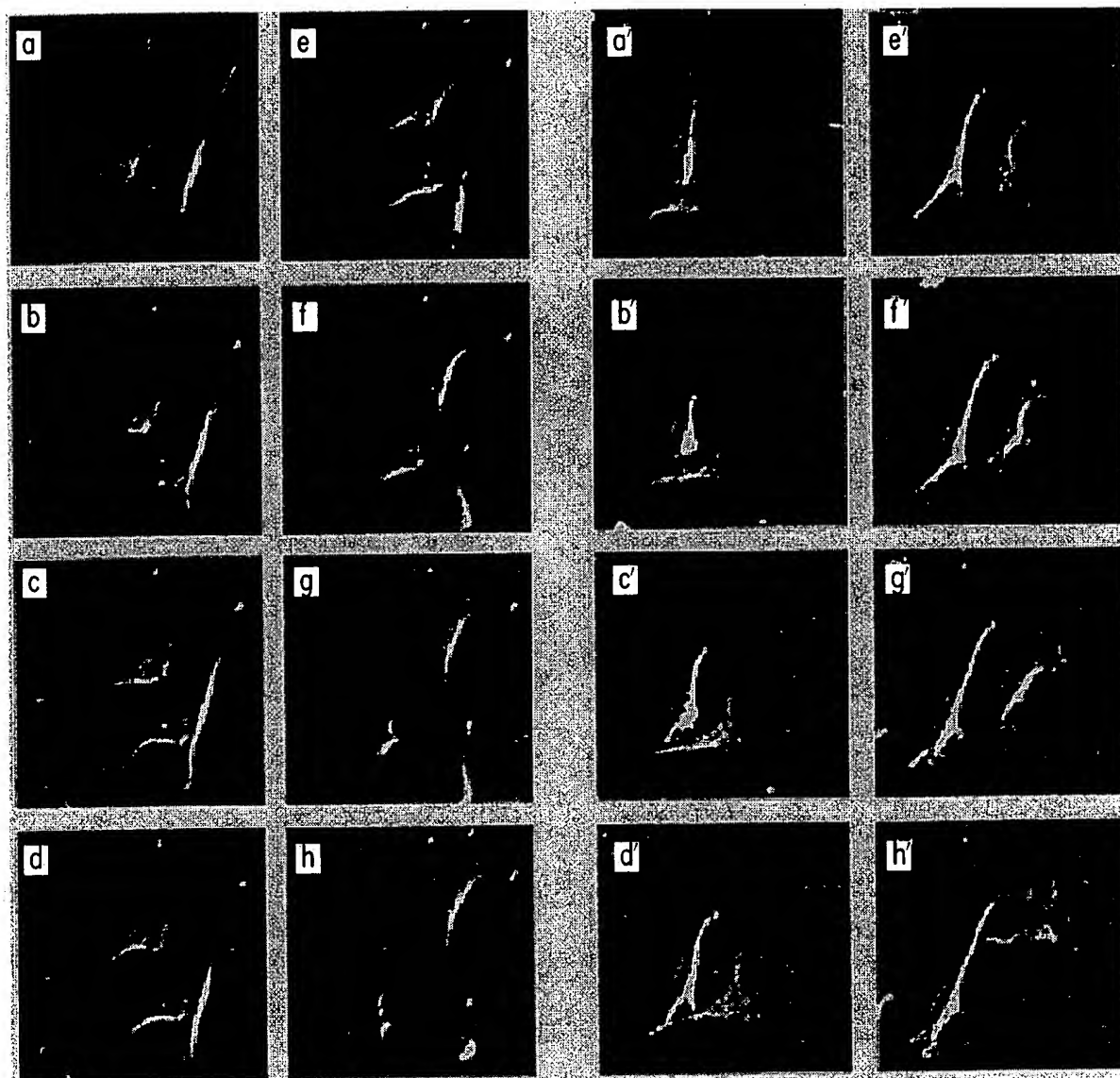
Fig. 16A

Fig. 16B
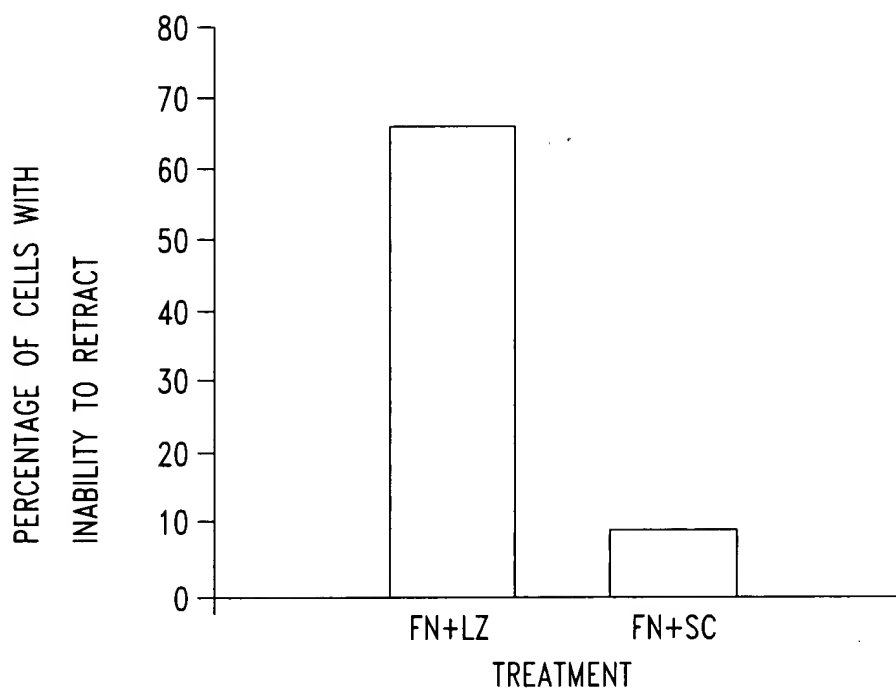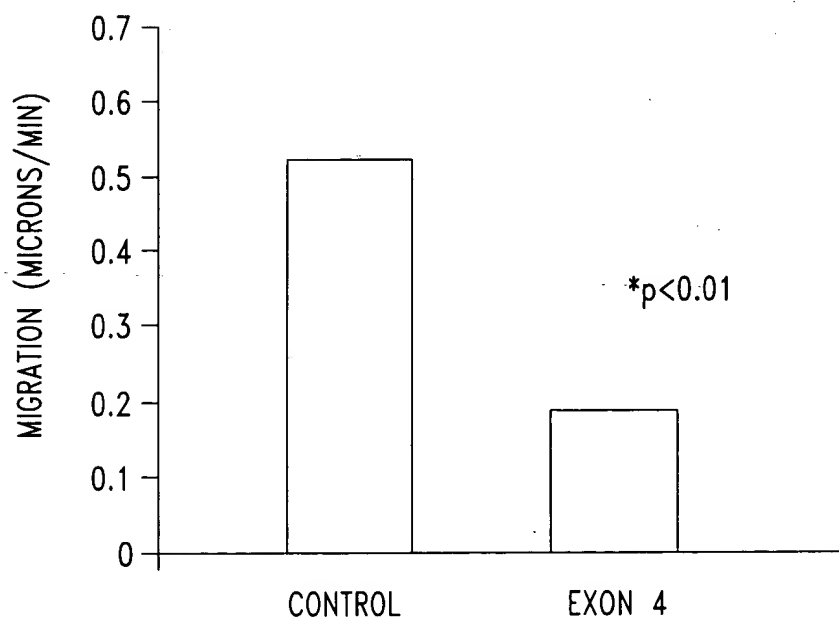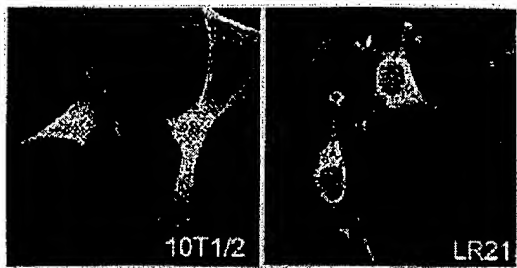
Fig. 16C

Fig. 16D

Fig. 17A
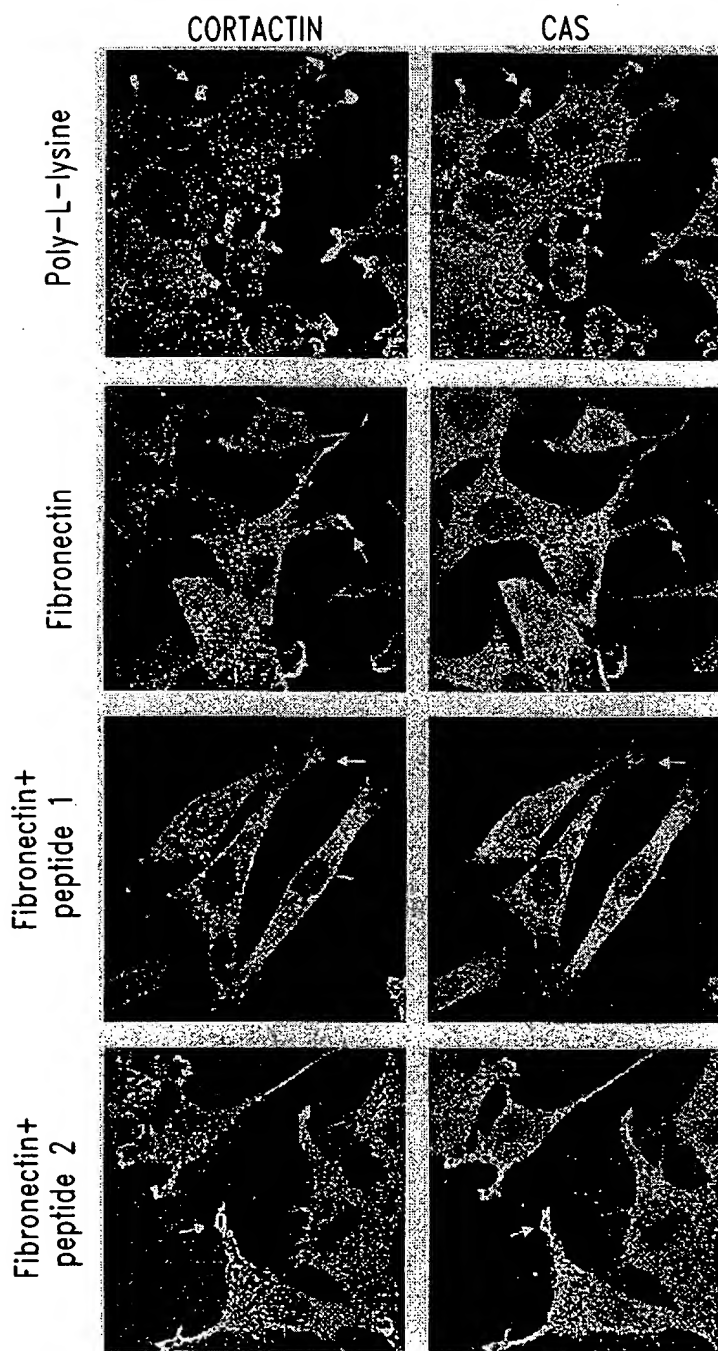
Fig. 17B
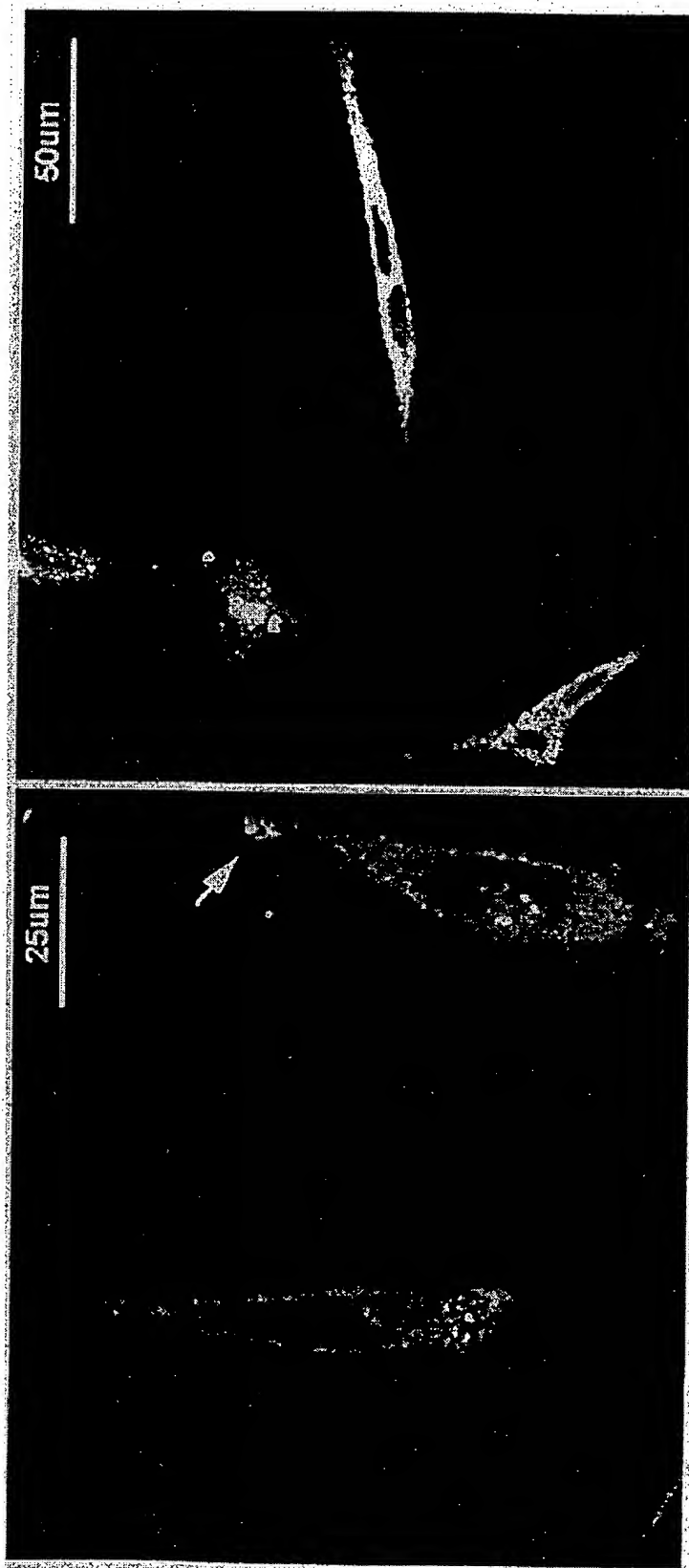
Fig. 18
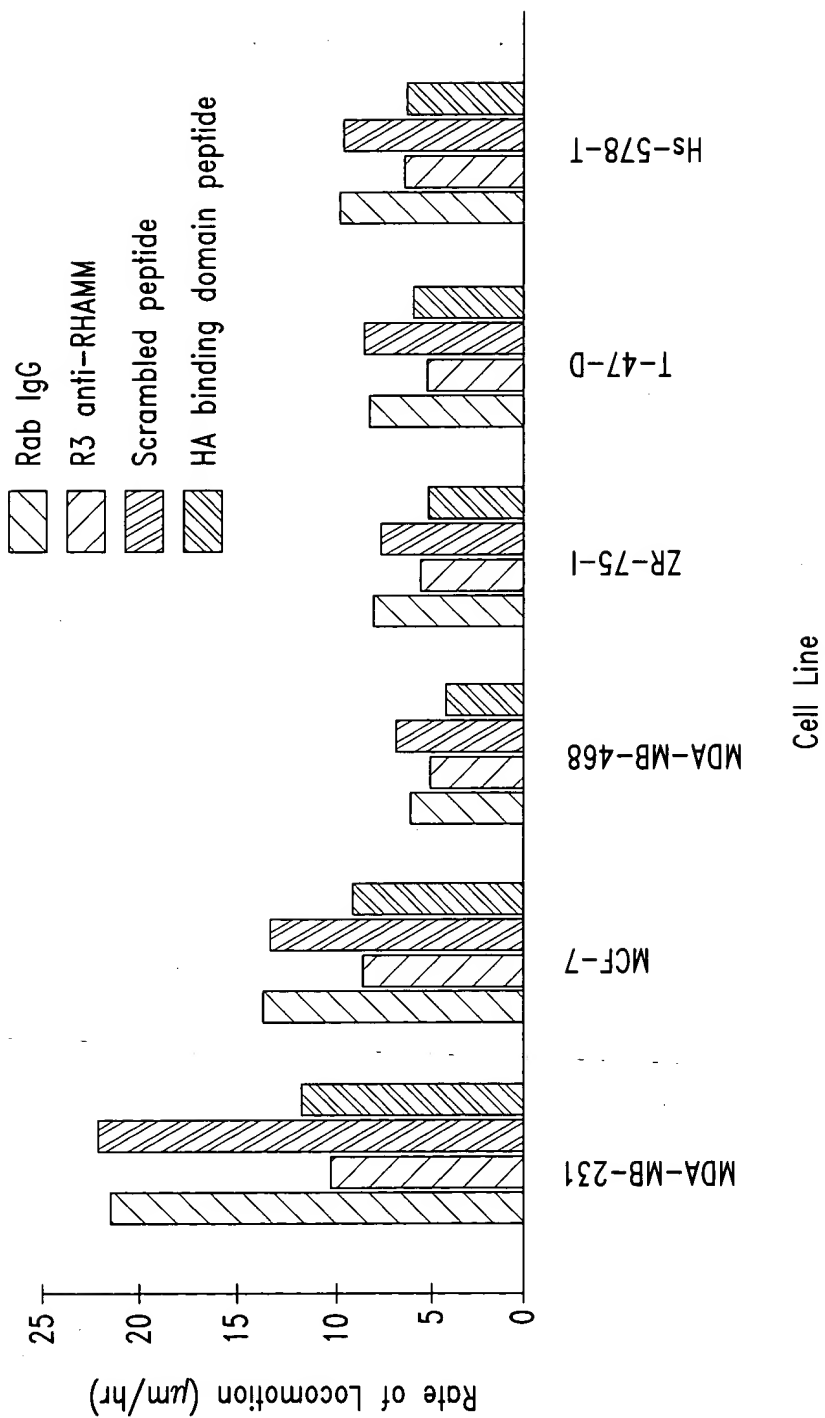
Fig. 19
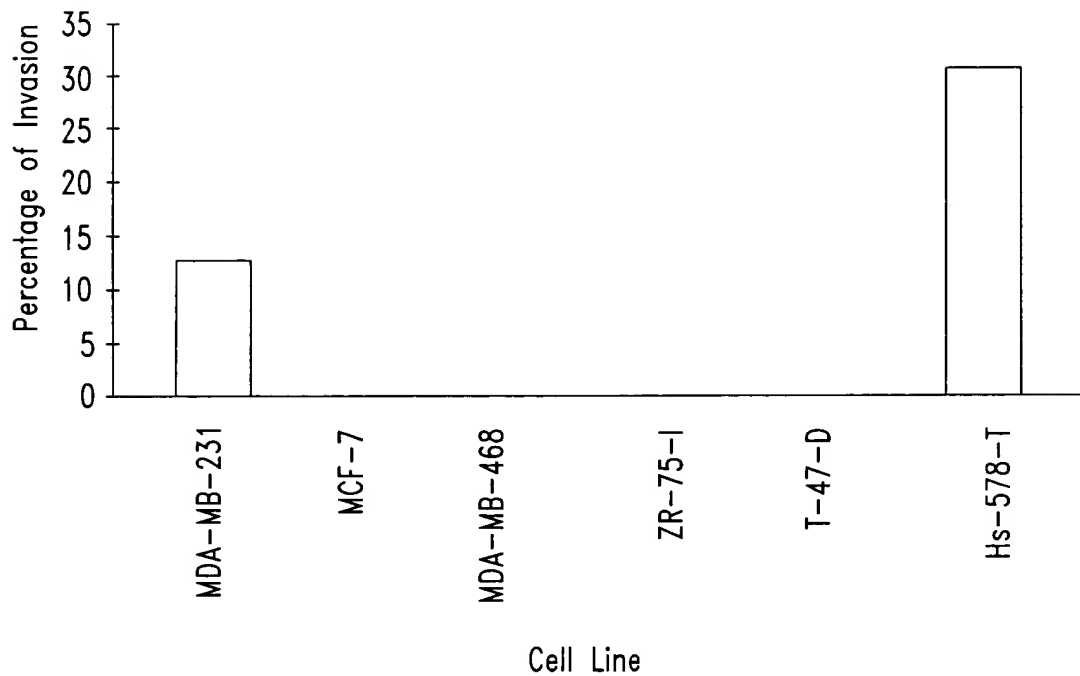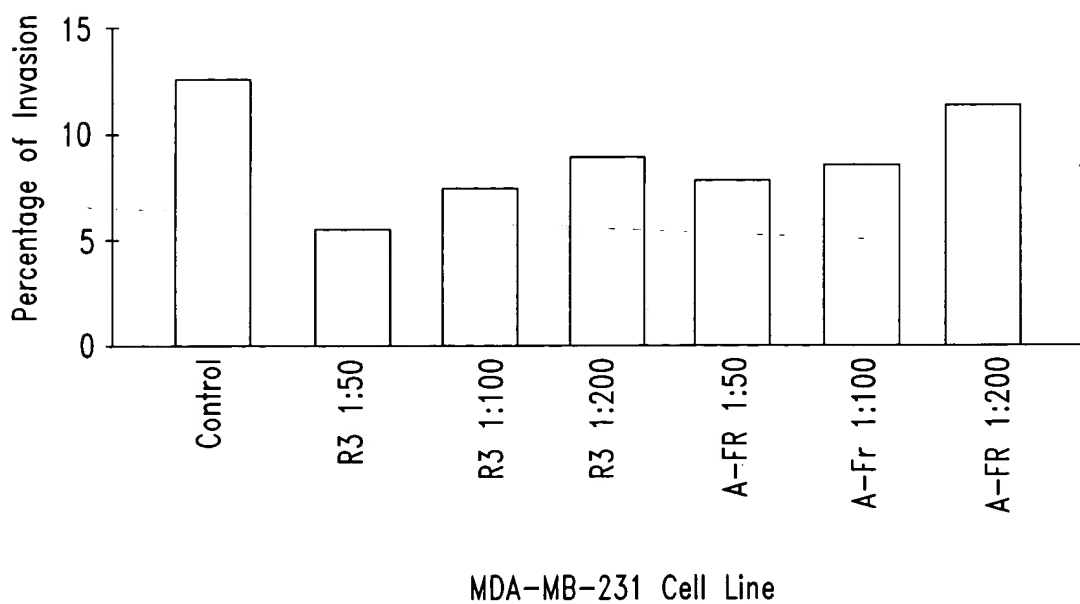
Fig. 20A

Fig. 20B
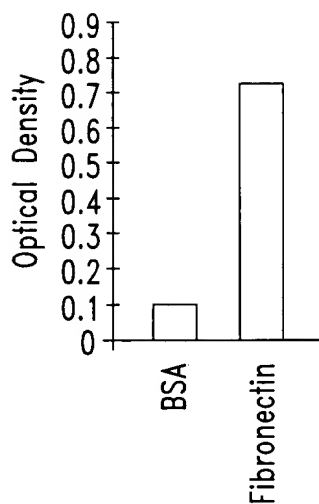
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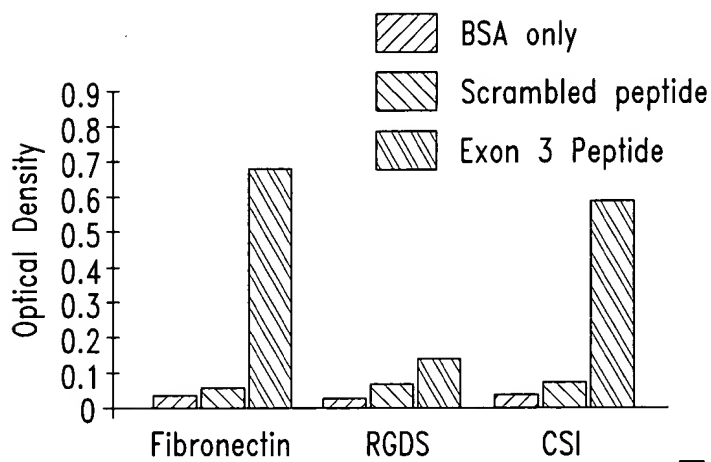
Fig. 21B
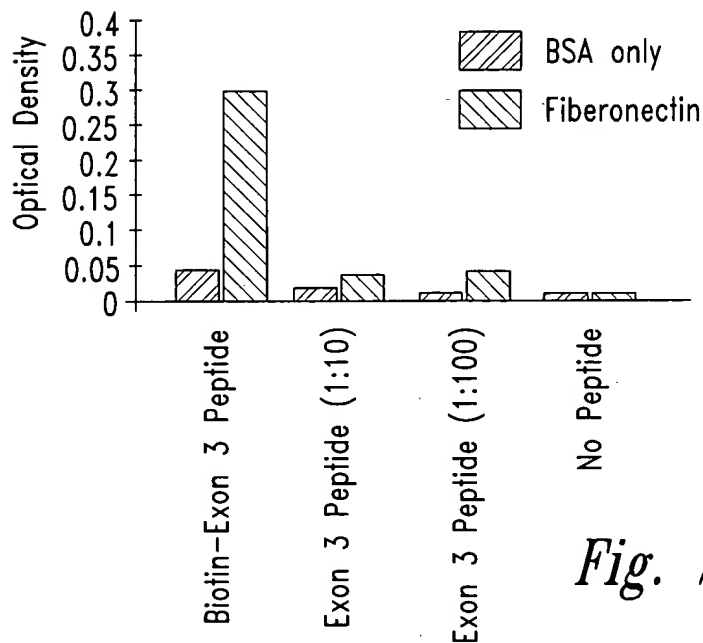
Fig. 21C
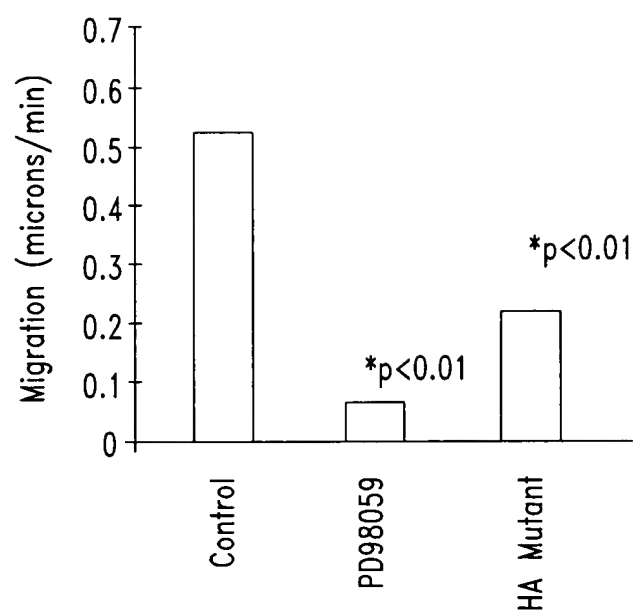
Fig. 22
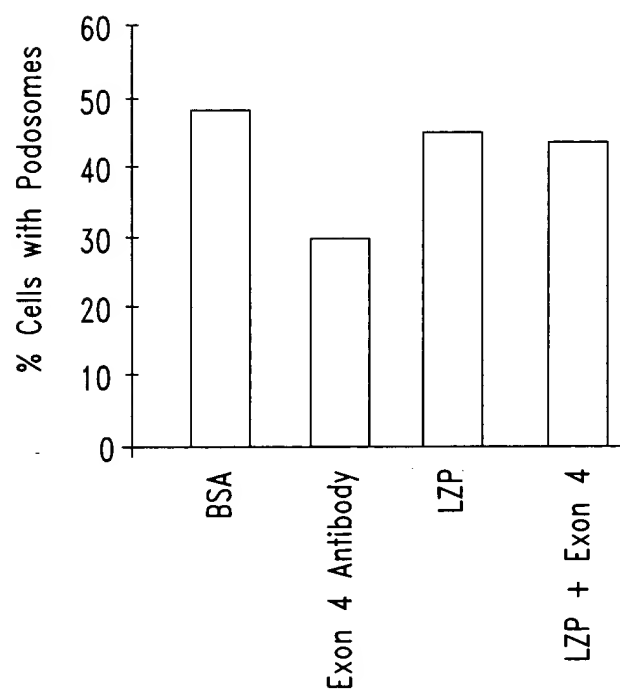
Effects of Exon4 Antibody and LZP
on the Podosome Formation of LR21

Fig. 24A

Exon4 HA-tag Merge

Fig. 23A

Cortactin FN-TR Overlay

Fig. 23B

Fig. 24B

Anti-RHAMM (exon3)

IgG control

Stromelysin I
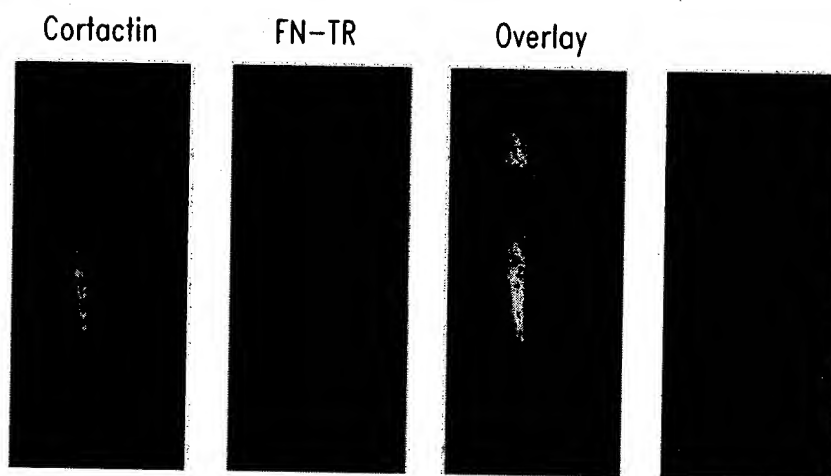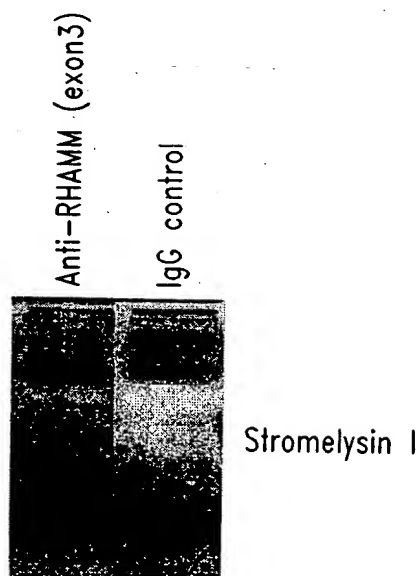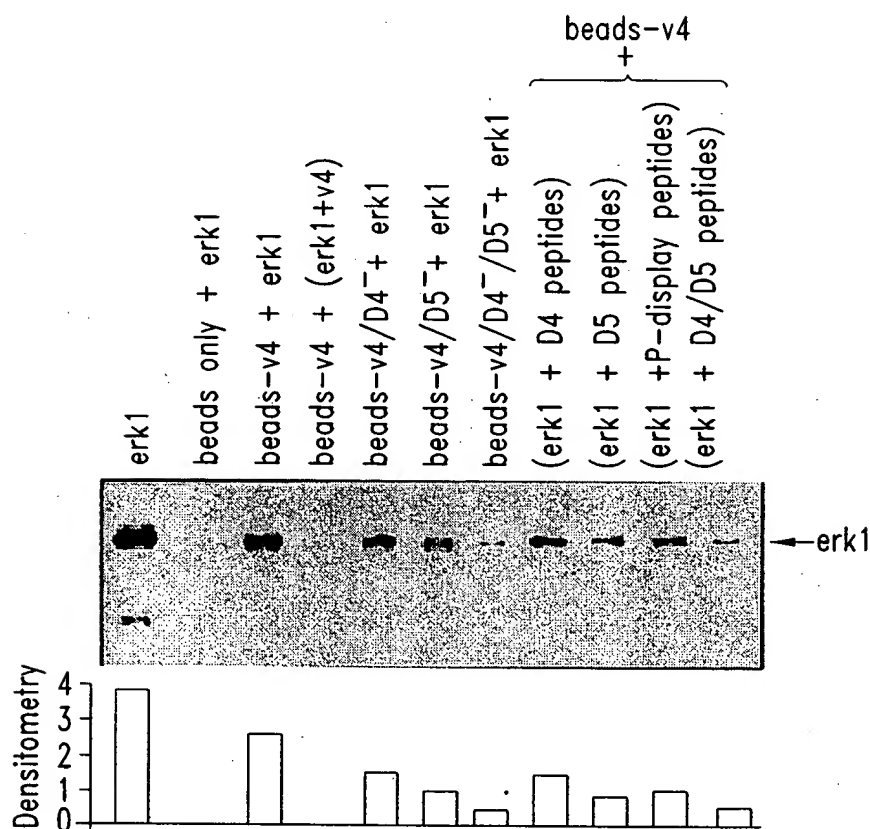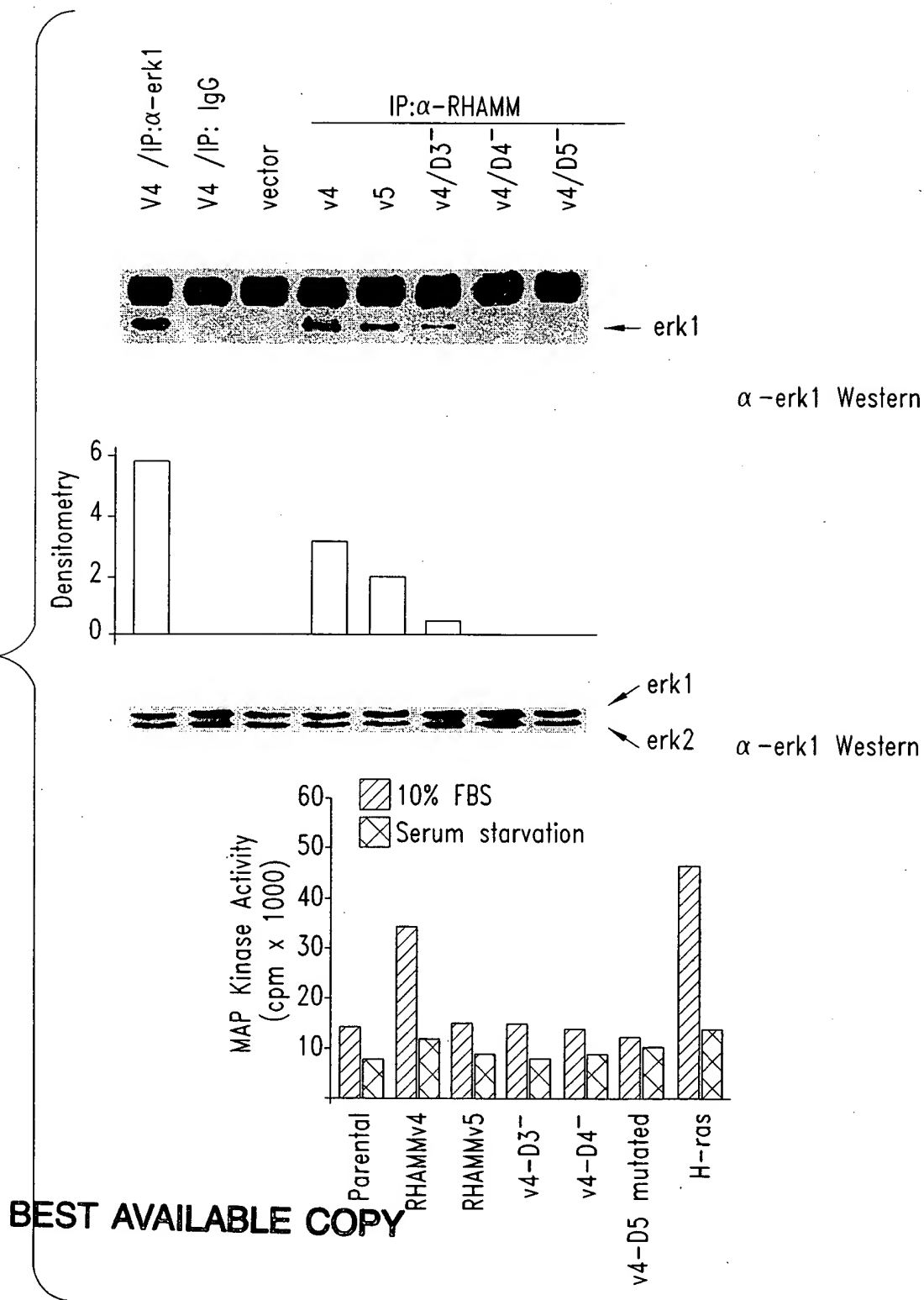
Fig. 25A

Fig. 25B

Fig. 25C

A: RGGGRGRRR
B: RGGGRGGRR
C: RGGGRGGGR
D: RGGGGGGGR
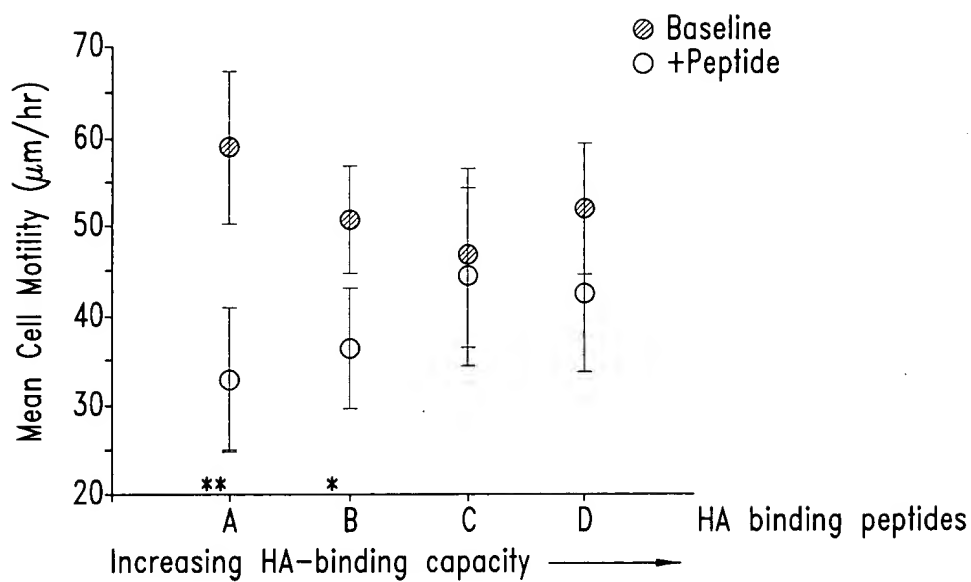
Fig. 26A

Fig. 26B
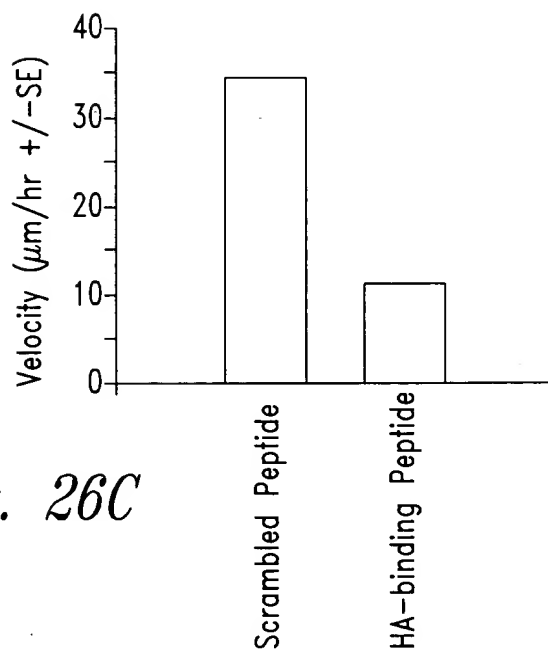
Fig. 26C
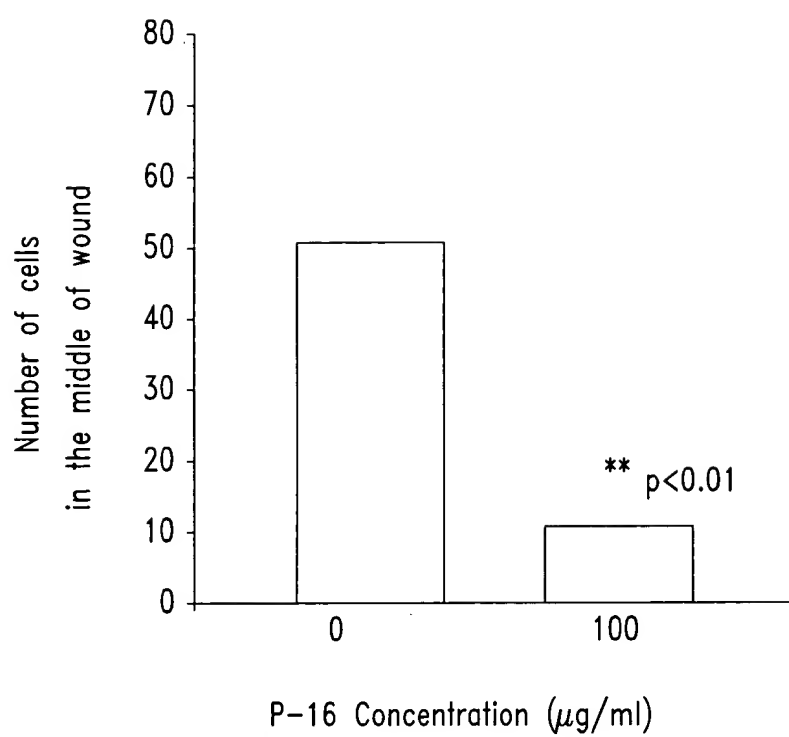
Fig. 27
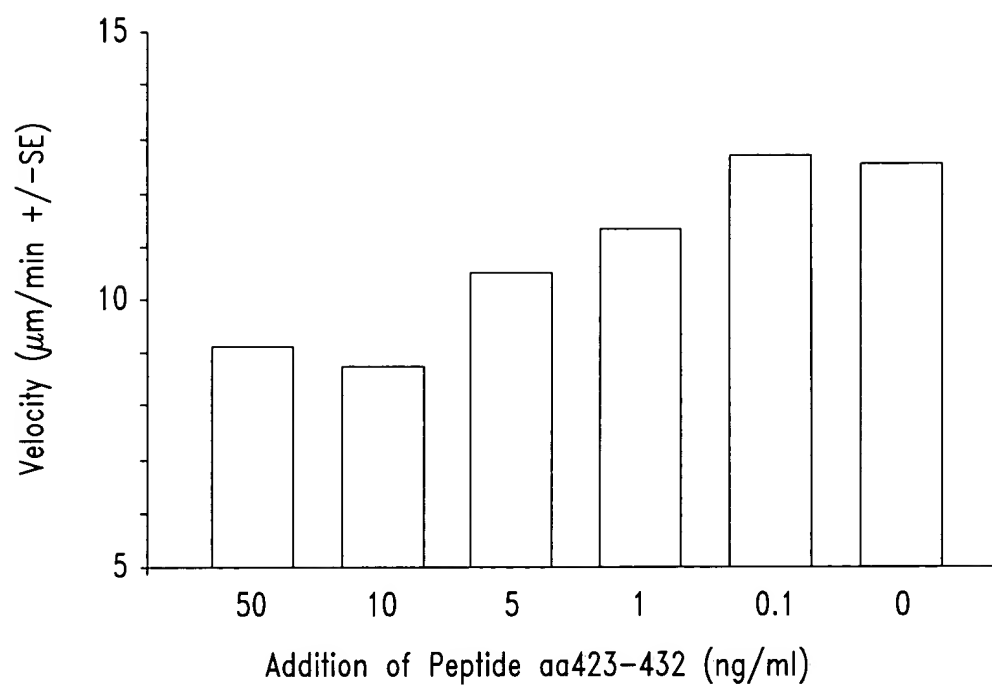
Fig. 28
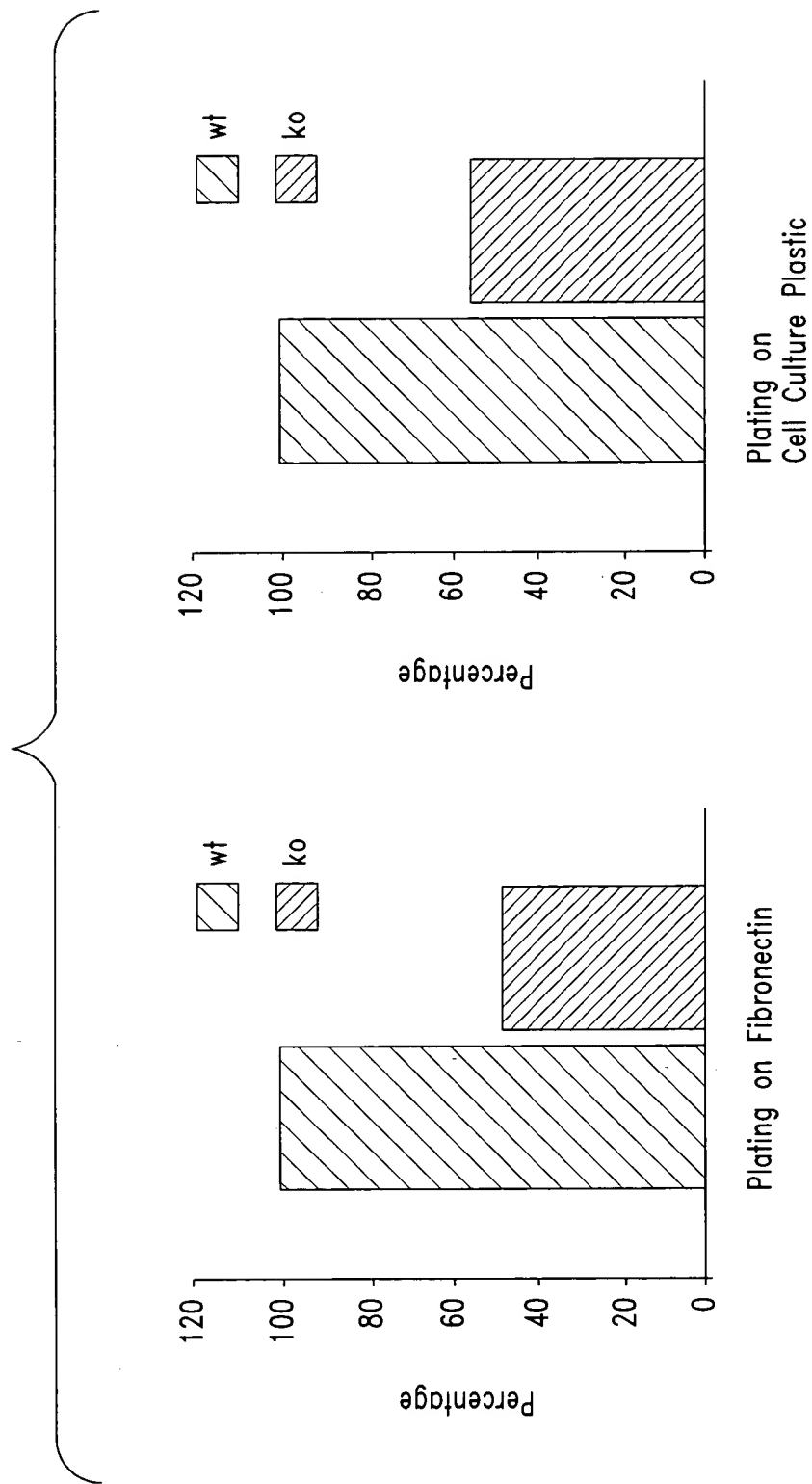
Fig. 29

Fig. 30

Random Cell Migration 2h after Plating

wt

ko

Fig. 31

Fig. 32

Fig. 33

Fig. 34

*p<0.05

Glucosaminidase Activity
(μU/20μl sample)

200

100

0

Bleomycin

Bleomycin +
Scrambled Peptide A

Bleomycin+
Peptide A

Fig. 35A

Control uninjured

4 days after injury

4 days + HA-binding peptide

4 days + scrambled peptide

Collagen α1

GAPDH
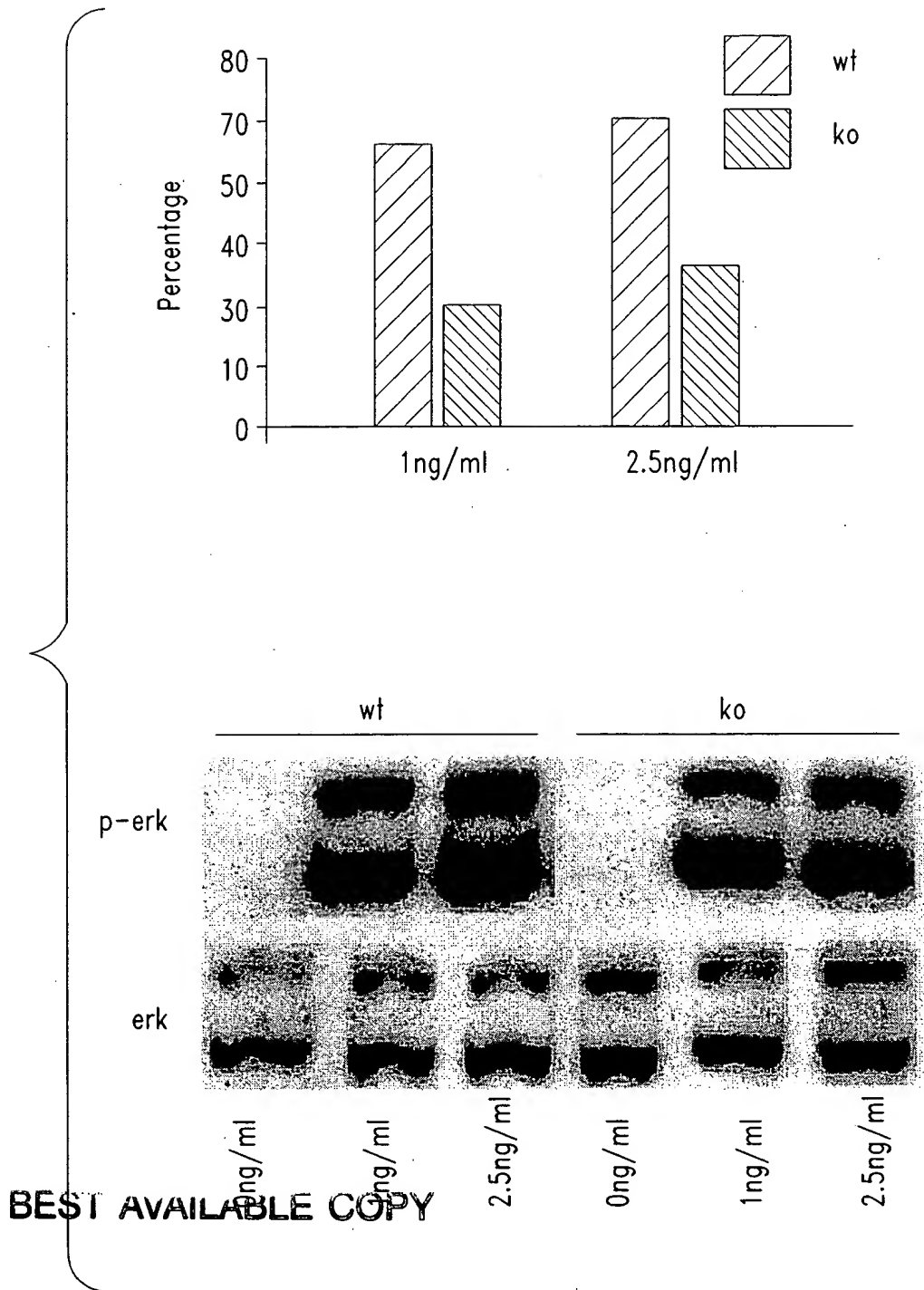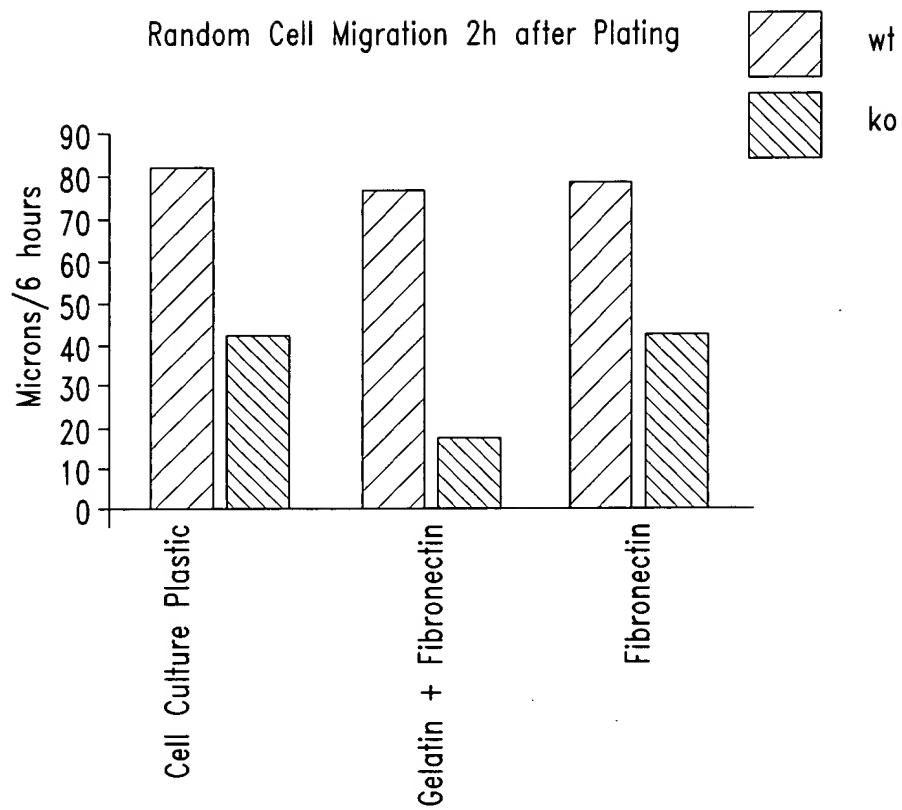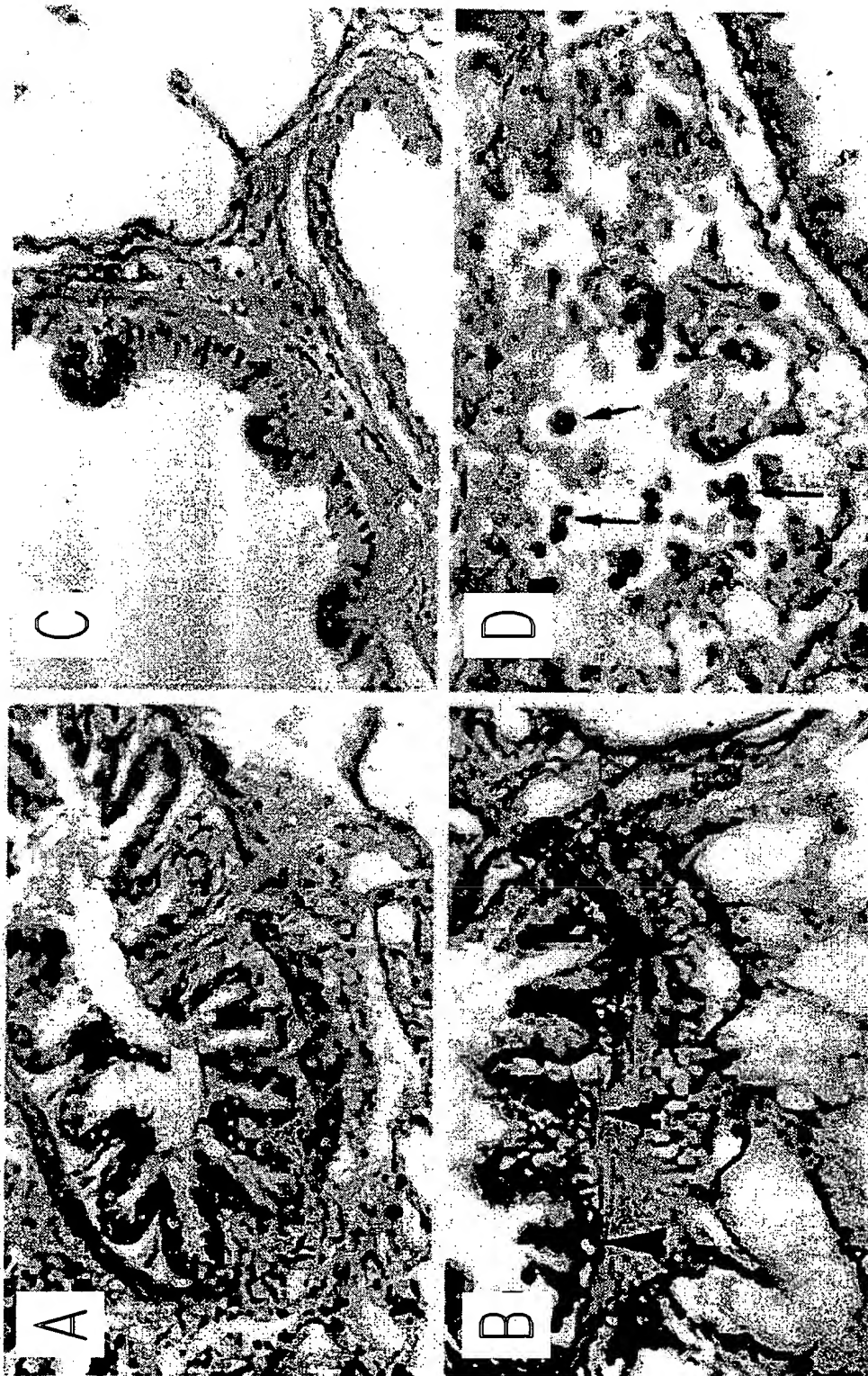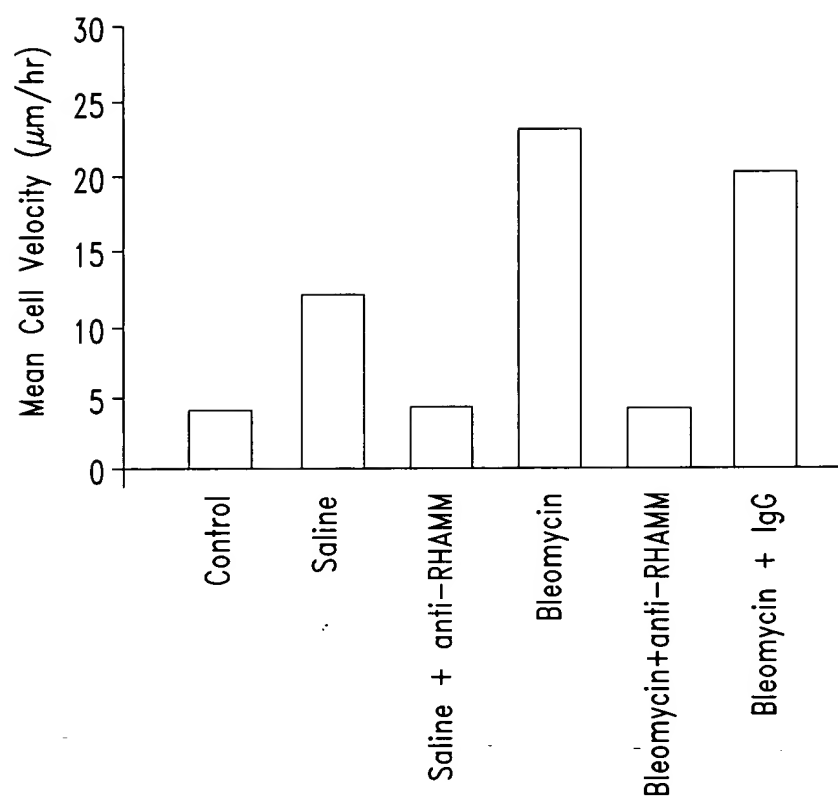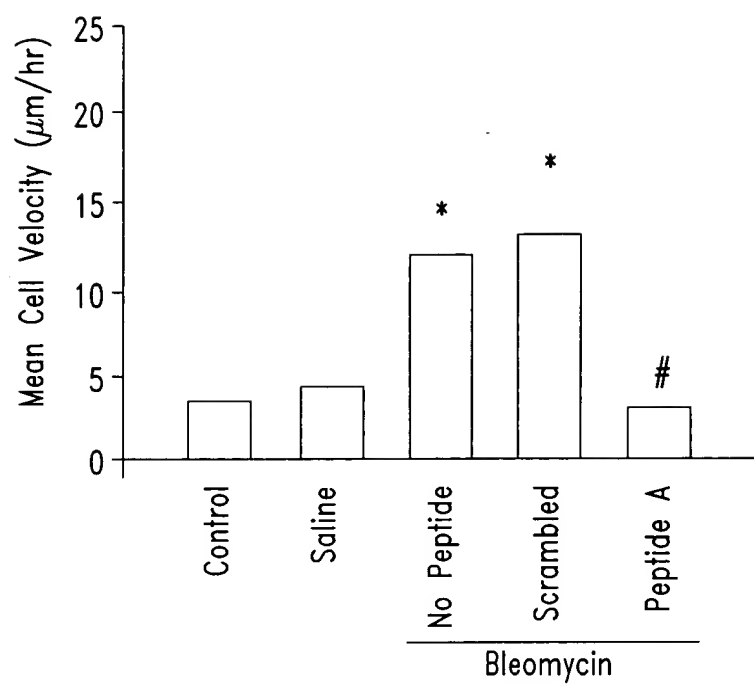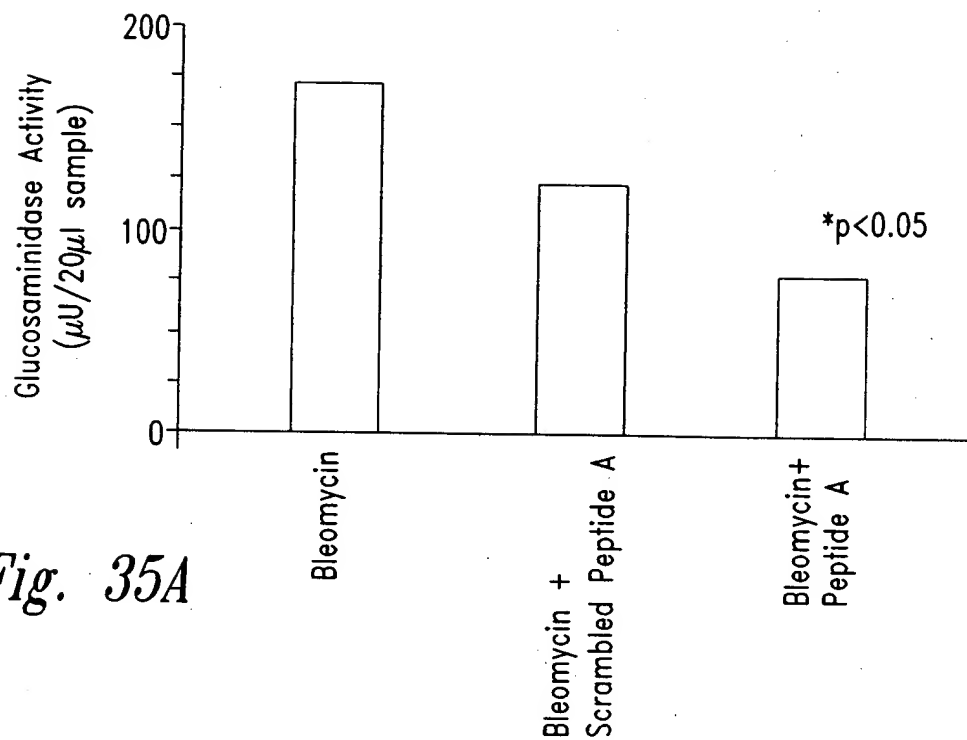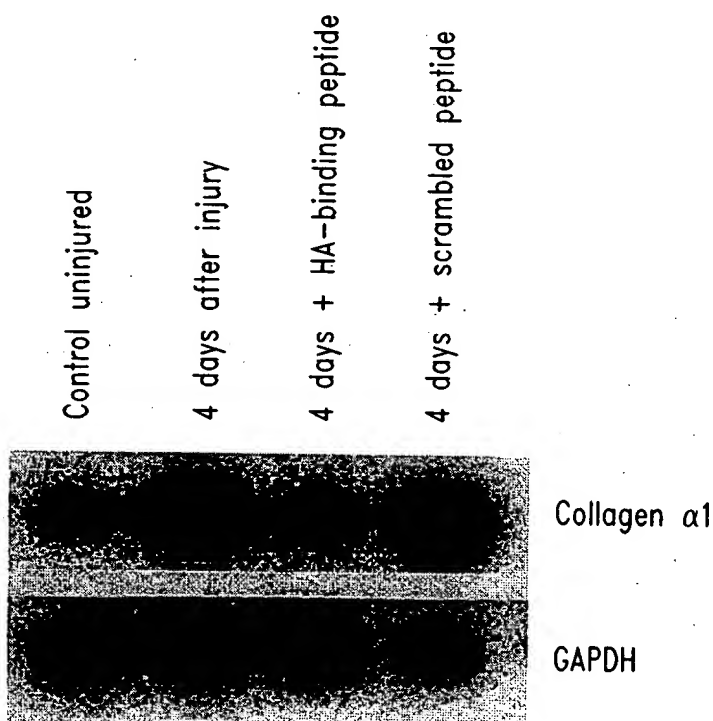
Fig. 35B
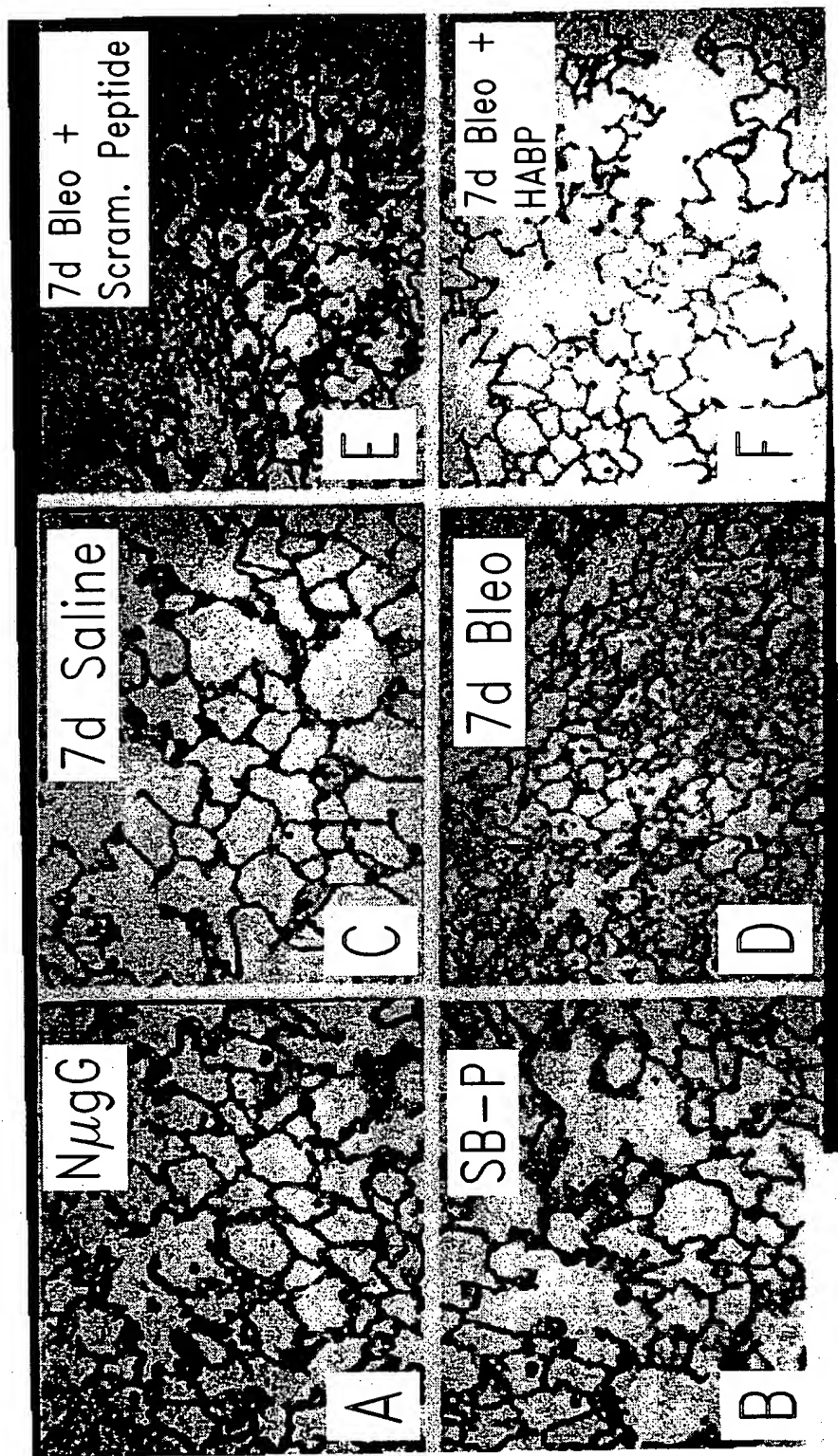
Fig. 36

Patient	% of total X4+ cells	% of total V5+ cells	Neutrophils			Monocytes/macrophages			T cells		
			% of total cells	% of X4+ cells	% of V5+ cells	% of total cells	% of X4+ cells	% of V5+ cells	% of total cells	% of X4+ cells	% of V5+ cells
W.H.	ND	50.7	70.5	81.2	ND	21.8	87.1	66.4	6.7	11.7	13.0
M.T.	74.6	20.7	80.7	ND	9.9	11.2	89.6	9.0	<2.0	<2.0	ND
L.S.	43.9	34.4	ND	8.5	ND	8.5	ND	53.8	20.4	5.3	<2.0
S.M.	67.6	4.0	67.3	80.9	ND	ND	ND	ND	3.0	10.0	<2.0
M.M.	19.2	19.6	25.2	ND	ND	ND	ND	ND	2.7	4.5	8.0
D.D	35.7	31.2	40.7	99.3	ND	ND	ND	ND	6.9	<2.0	9.9
P.B. (r)	77.4	71.8	ND	ND	ND	9.2	99.8	88.3	4.4	13.0	33.2
P.B. (l)	85.0	82.3	ND	ND	ND	12.8	99.4	58.3	3.4	11.0	30.2
S.L.	51.6	45.5	61.7	92.1	77.2	8.8	73.4	85.6	24.0	6.0	9.0
R.C.	10.6	6.7	54.1	63.8	13.8	5.6	50.3	43.9	6.3	8.5	11.9
N.N.	27.9	10.3	44.1	54.6	21.4	3.5	77.1	49.4	6.8	33.1	22.2
M.G	85.48	84.63	86.7	99.6	99.5	5.52	98.7	98.9	6.36	4.8	7.6

- ND - non-determined
- (r) - right knee
- (l) - left knee

Fig. 37
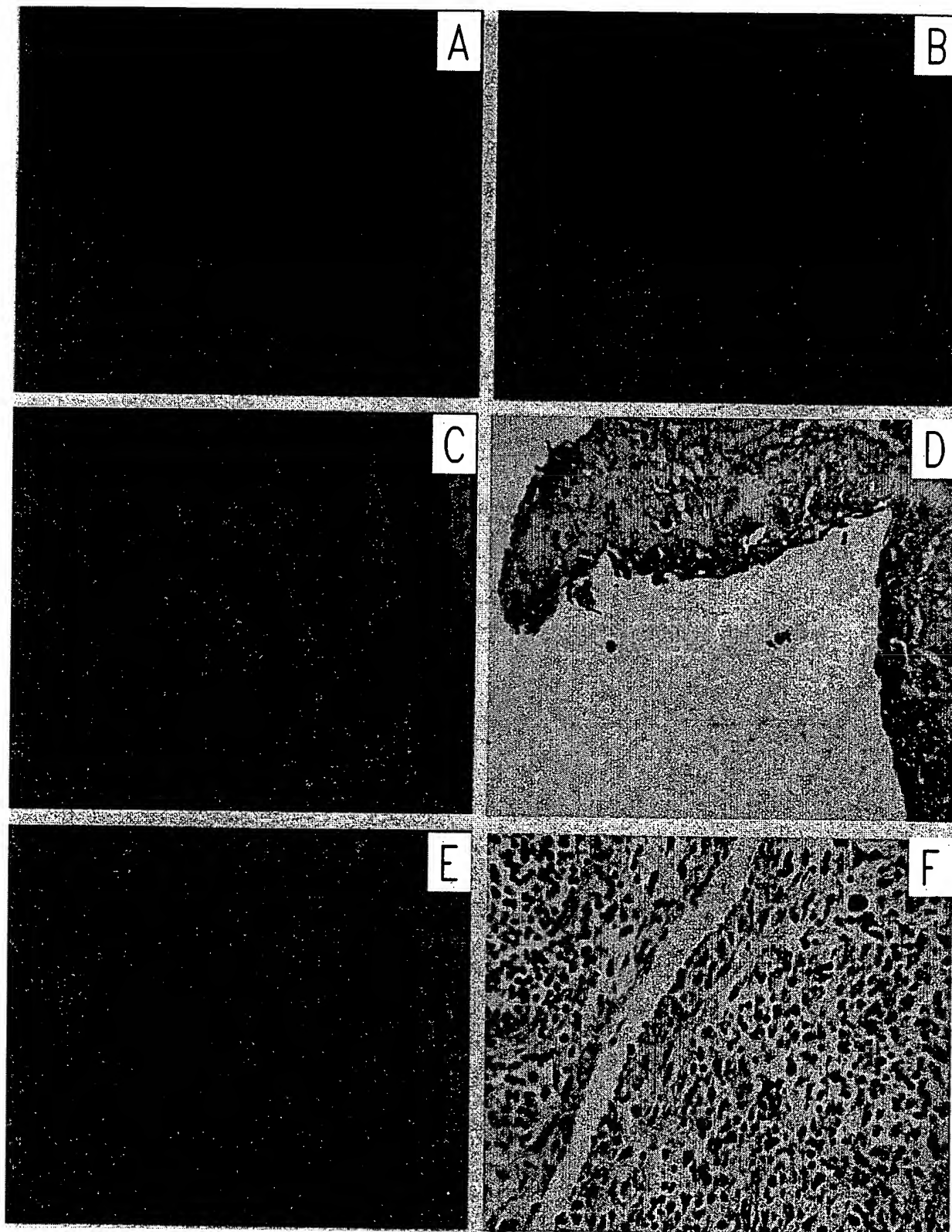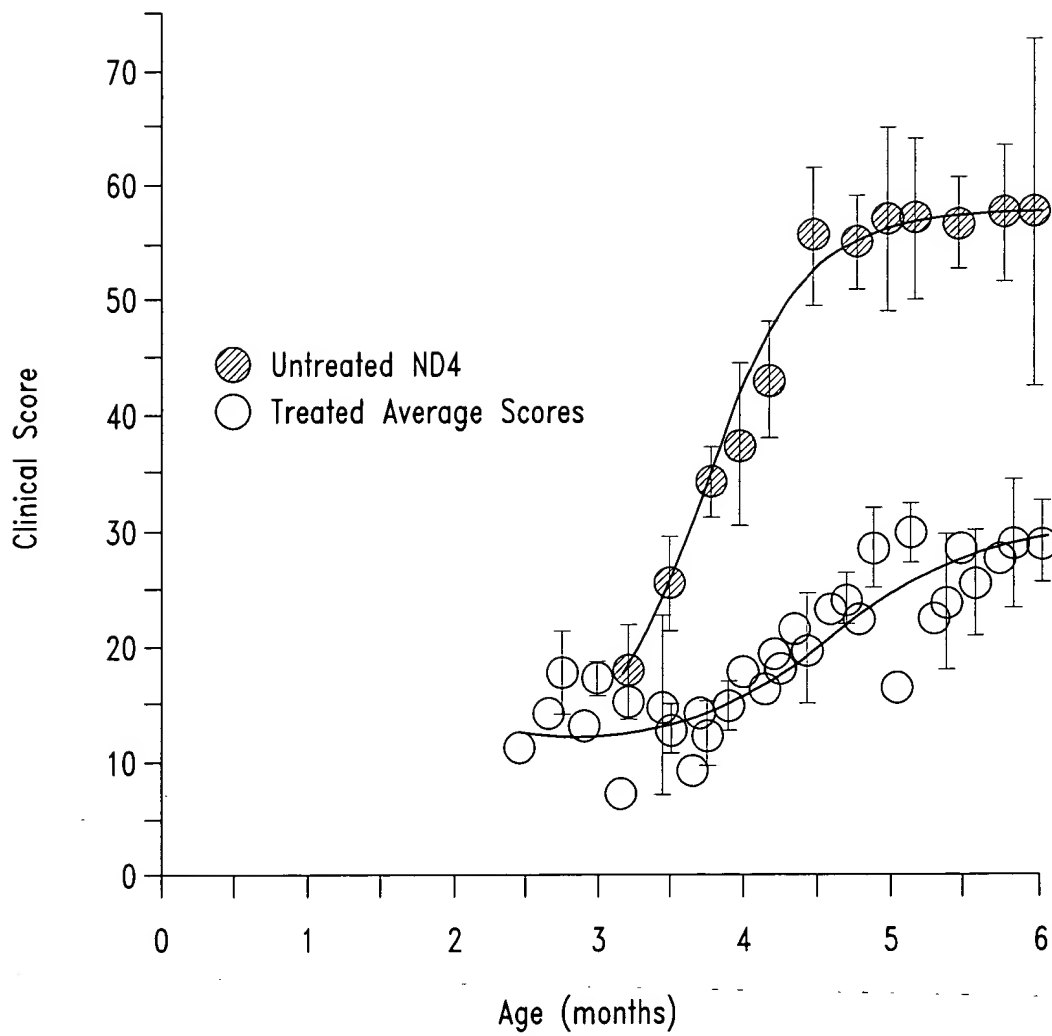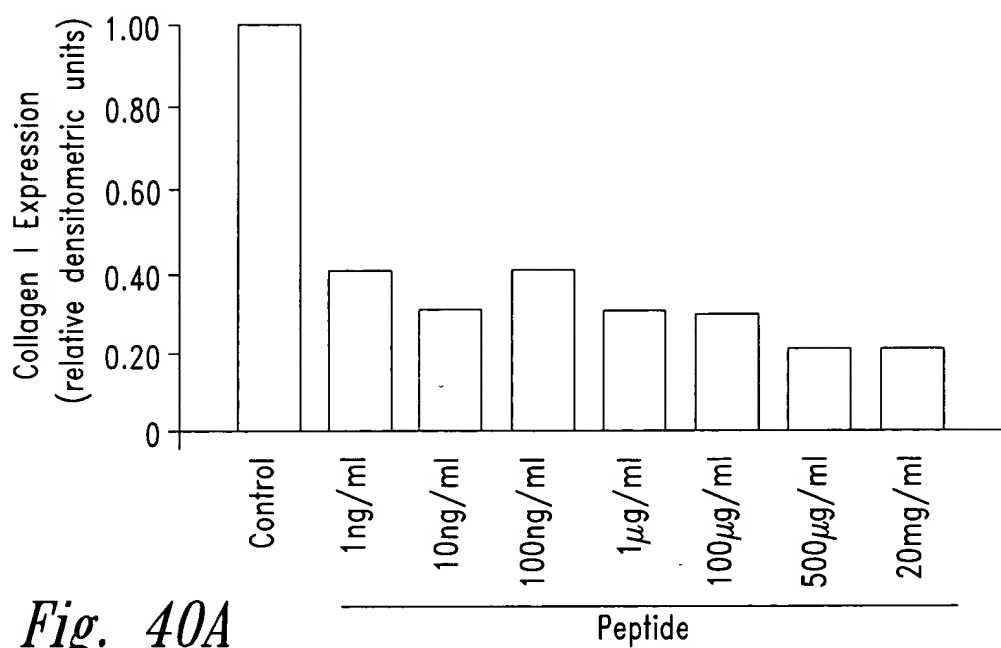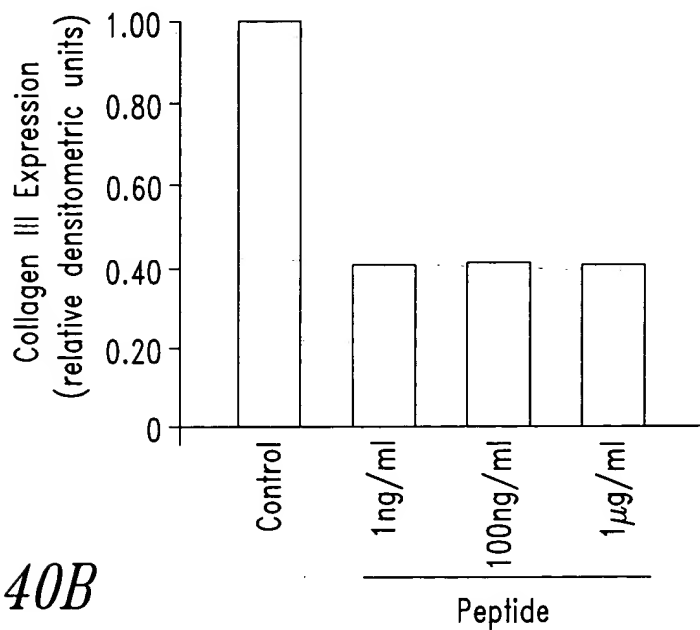
Fig. 38

Fig. 39

Fig. 40A

Fig. 40B

Fig. 41

Fig. 42

Cell lines: NbE   MC2   Fb2
Invasivity: 0   ++   ++++

250
98
64 — 54kDa
50

36

30

Fig. 43A

AT-1   MatLyLu

NbE   MC2   Fb2

Fig. 43B

Fig. 44A

Fig. 44B

NZB/W Mice (N=10/group)

-◆- Noninjected
-■- *Injected (8 wks)
-▲- *Injected (16 wks)
-✕- *Injected (24 wks)

*5 mg RHAMM Antagonist/kg
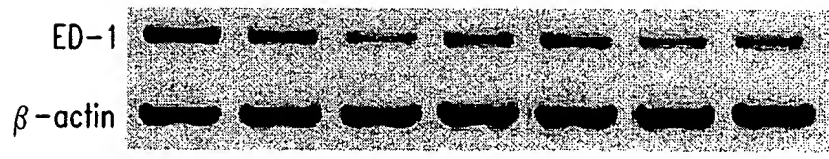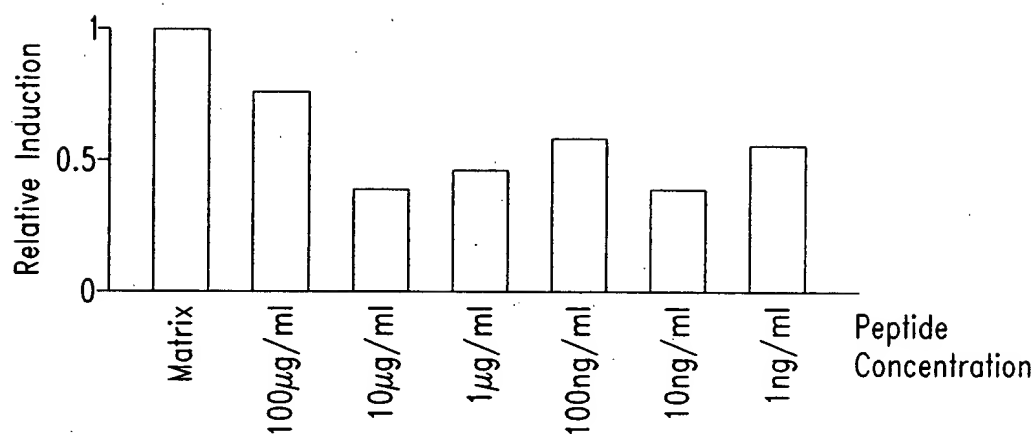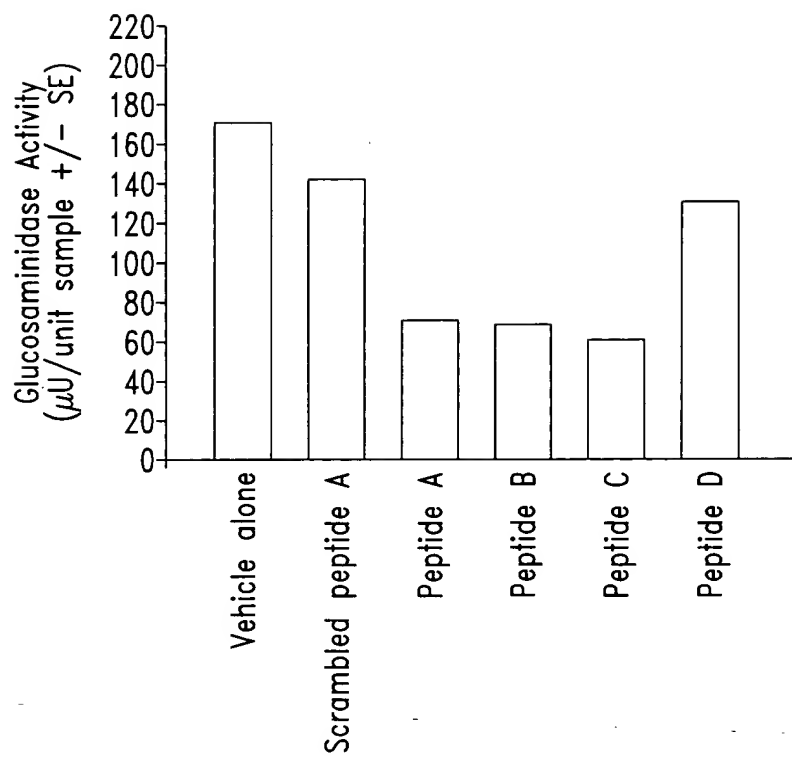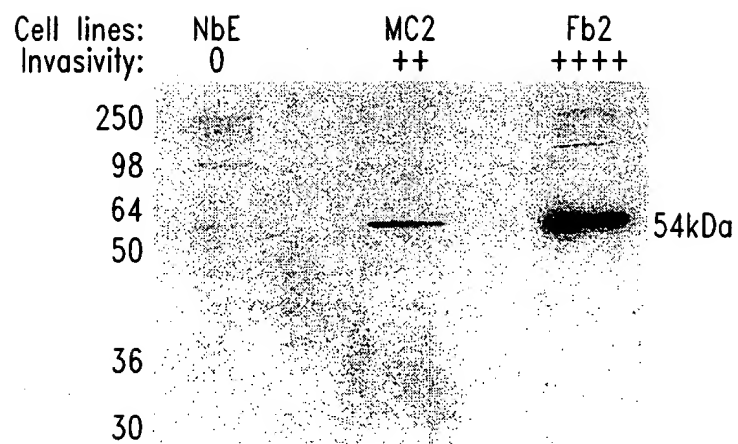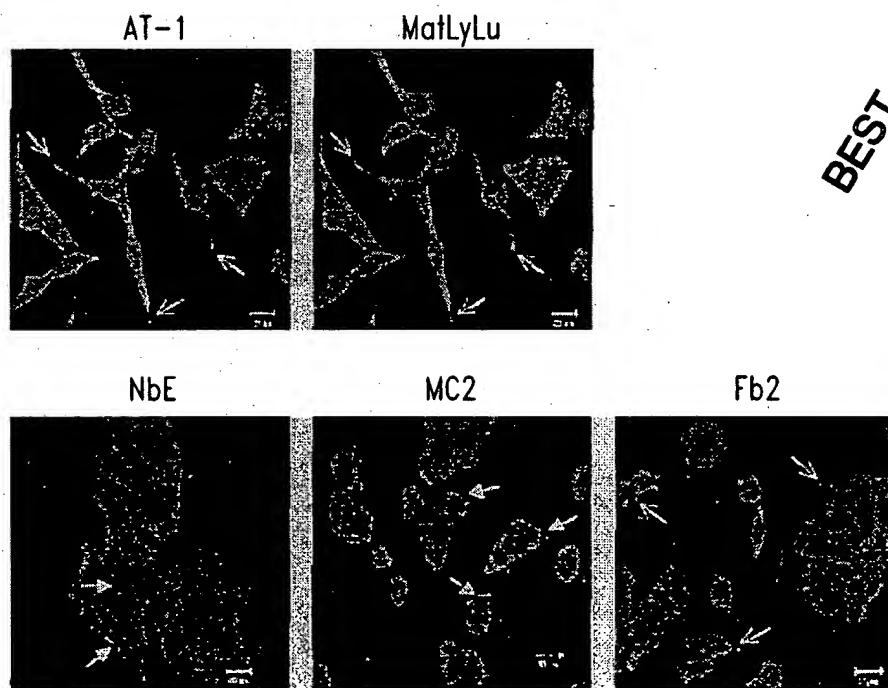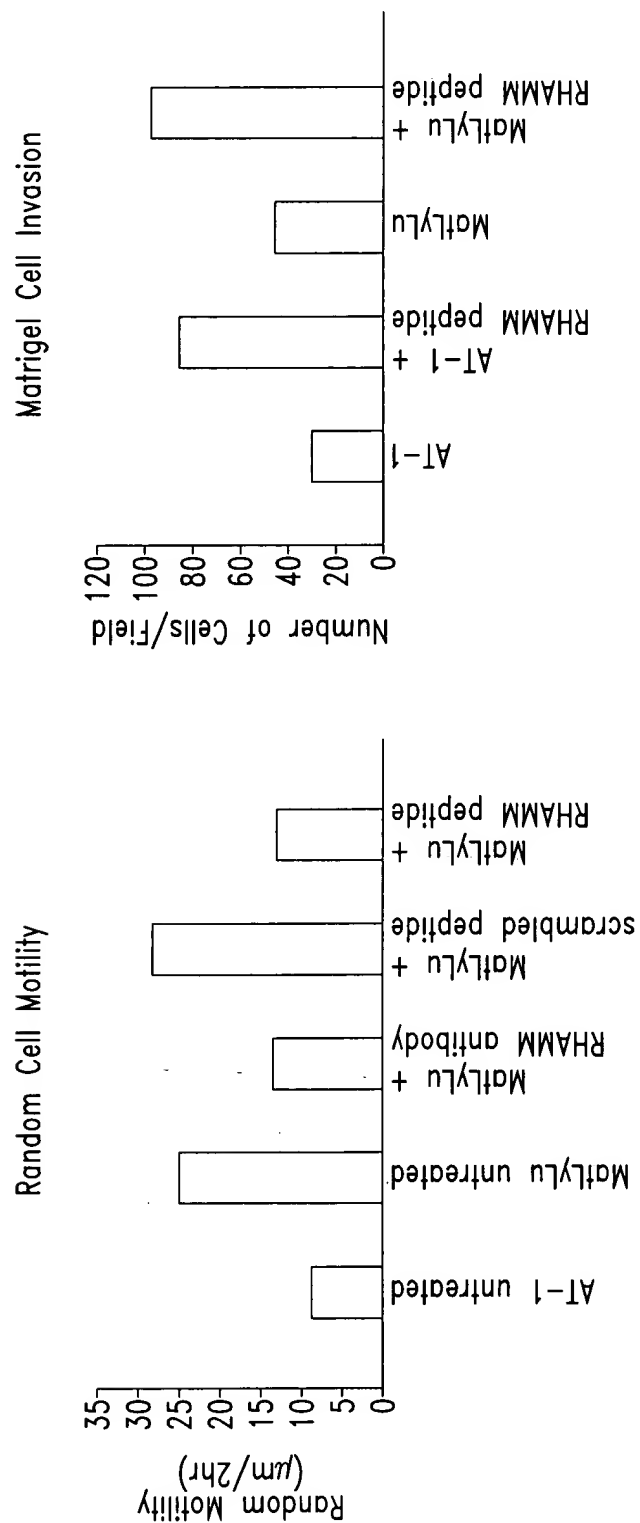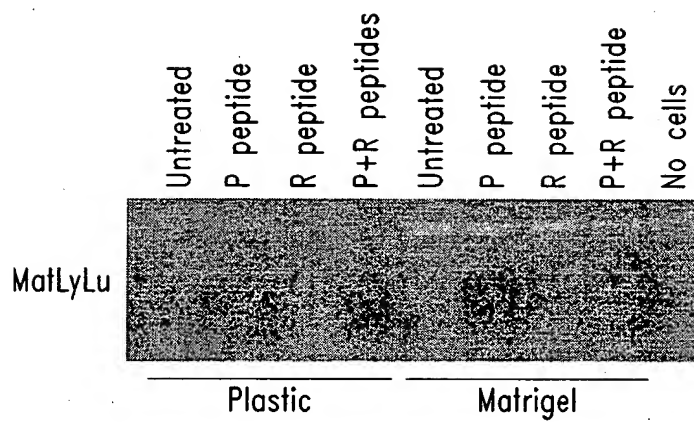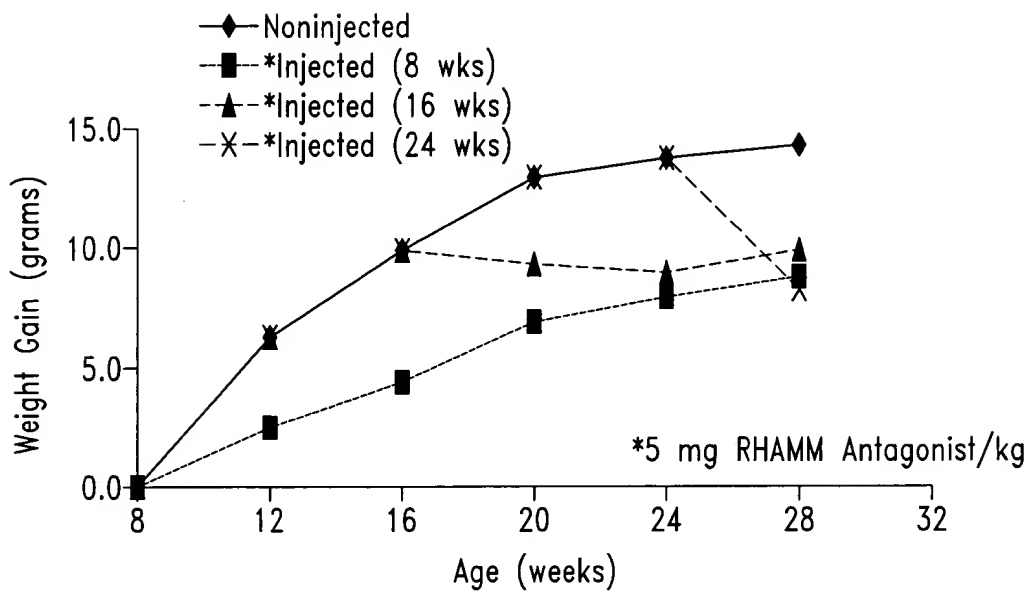
Note: This effect is not being seen with NOD mice

Fig. 45
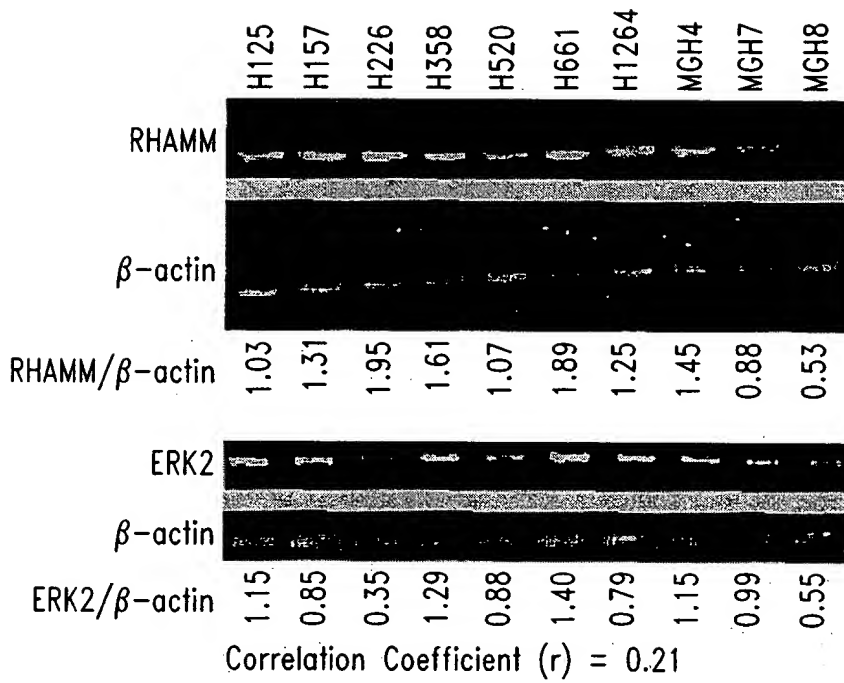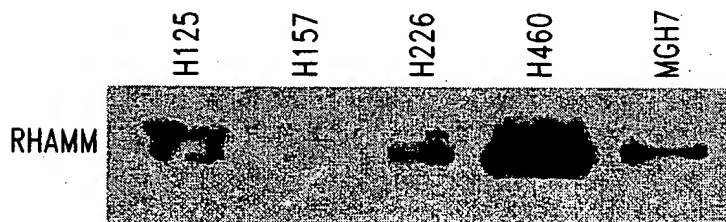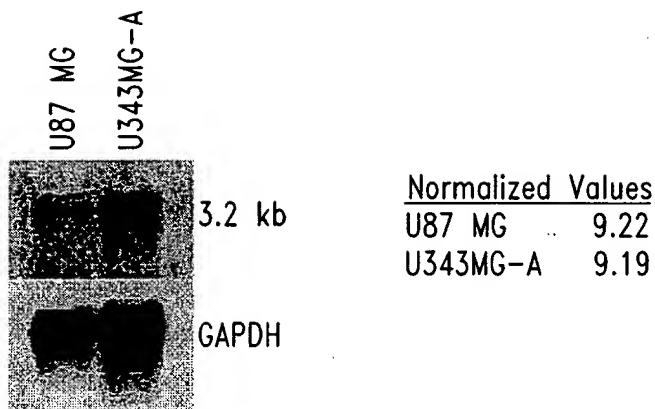
RHAMM

β—actin

RHAMM/β—actin | 1.03 | 1.31 | 1.95 | 1.61 | 1.07 | 1.89 | 1.25 | 1.45 | 0.88 | 0.53

ERK2

β—actin

ERK2/β—actin | 1.15 | 0.85 | 0.35 | 1.29 | 0.88 | 1.40 | 0.79 | 1.15 | 0.99 | 0.55

H125 H157 H226 H358 H520 H661 H1264 MGH4 MGH7 MGH8

Correlation Coefficient (r) = 0.21

Fig. 46A

RHAMM

H125 H157 H226 H460 MGH7

Fig. 46B

Normalized Values
U87 MG   9.22
U343MG-A   9.19
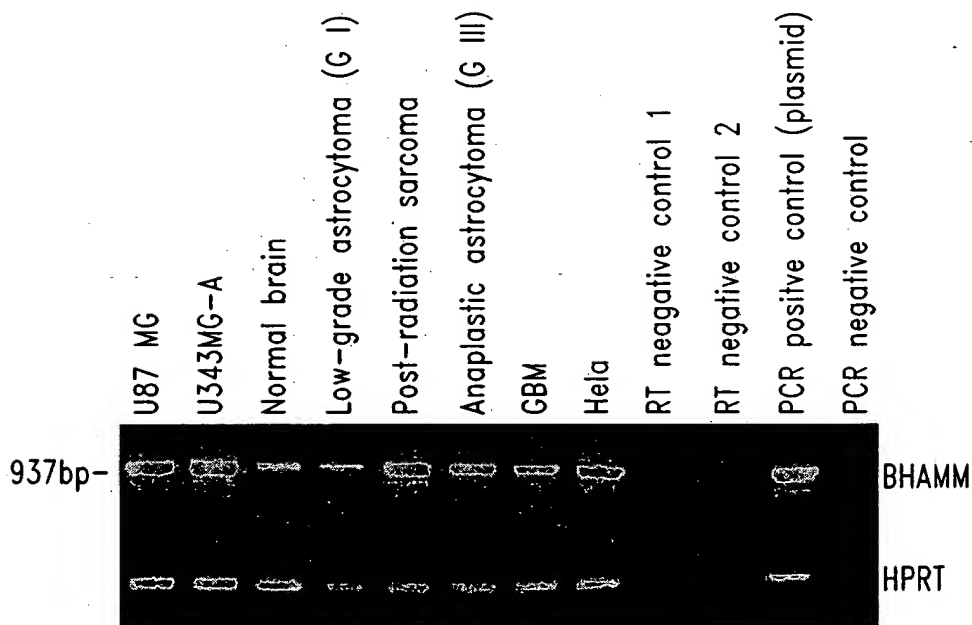
Fig. 47A

Fig. 47B
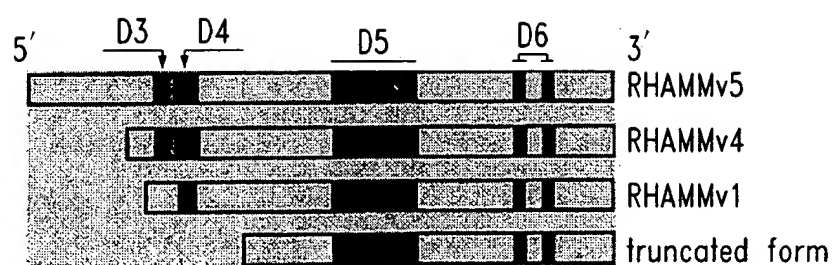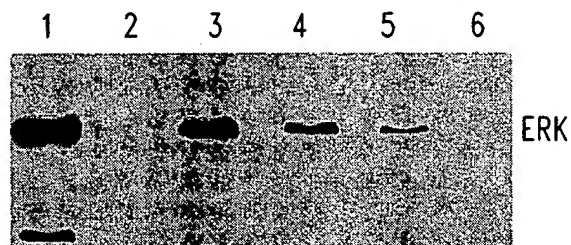
Fig. 48A

Fig. 48B

RHAMM binding protein cDNA (RABP) (partial)

```
GAA TTC GCG GCG GCG TCG ACC AAC AAG CCC CCT GCT GTT TCC CCG GGG
 E   F   A   A   A   S   T   N   K   P   P   A   V   S   P   G
GTG GTC TCC CCA ACC TTT GAA CTT ACA AAT CTT CTA AAT CAT CCT GAC
 V   V   S   P   T   F   E   L   T   N   L   L   N   H   P   D
CAT TAT GTA GAA ACA GAG AAC ATT CAG CAT CTC ACA GAC CCG GCT CTA
 H   Y   V   E   T   E   N   I   Q   H   L   T   D   P   A   L
GCA CAT GTG GAT AGA ATA AGC GAA GCC CGG AAA CTG AGT ATG GGA TCT
 A   H   V   D   R   I   S   Q   A   R   K   L   S   M   G   S
GAT GAT GCT GCC TAC ACA CAA GCT CTG CTG GTG CAC CAG AAG GCC AGG
 D   D   A   A   Y   T   Q   A   L   L   V   H   Q   K   A   R
ATG GAA CGG CTT CAA AGA GAG CTC GAG ATG CAA AAG AAA AAG CTG GAT
 M   E   R   L   Q   R   E   L   E   M   Q   K   K   K   L   D
AAA CTC AAA TCT GAG GTC AAT GAG ATG GAA AAT AAT CTA ACT CGA AGG
 K   L   K   S   E   V   N   E   M   E   N   N   L   T   R   R
CGC CTG AAG AGA TCA AAT TCC ATT TCC CAG ATA CCG TCA CTC GAA GAA
 R   L   K   R   S   N   S   I   S   Q   I   P   S   L   E   E
ATG CAG CAG TTG AGA AGT TGT AAT AGA CAA CTC CAG ATT GAC ATT GAC
 M   Q   Q   L   R   S   C   N   R   Q   L   Q   I   D   I   D
TTT GAC TGC TTA ACC AAA GAA ATT GCA TCT TTT TCA AGC CCG AGG ACC
 F   D   C   L   T   K   E   I   A   S   F   S   S   P   R   T
ACA TTT TAA CCC CAG CGC TAT TCA TAA CTT TTA TGA CAA TAT TGG ATT
 T   F   *
TGT AGG CCC TGT GCC ACC AAA ACC CAA AGA TCA AAG GTC CAC CAT CAA
AGG TCG ACG CGG
```

Fig. 49A

RABP — -4.2 kDa

— -2.8 kDa

Fig. 49B

Anti-RABP

IgG Control

Anti-RABP +RABP

115–
84–
62–
51–
38–

Fig. 49C

Fig. 49D

```
Human:    1 MSFPKAPLKRFNDPSGCAPSPGAYDVKTLEVLKGPVSFQKSQRFKQQKESKQNLNVDKDTTLPASARKVKSSESK

Human:   76 KESQKNDKDLKILEKEIRVLLQERGAQDRRIQDLETELEKMEARLNAALREKTSLSANNATLEKQLIELTRTNEL

Human:  151 LKSKFSENGNQKNLRILSLELMKLRNKRETKMRGMMAKQEGMEMKLQVTQRSLEESQGKIAQLEGKLVSIEKEKI
              |+||||||||||||||||| || ||||||+||| ||+ | || ||||||||||||||||||
Mouse:    1              MRALSLELMKLRNKRETKMRSMMVKQEGMELKLQATQKDLTESKGKIVQLEGKLVSIEKEKI

Human:  226 DEKSETEKLLEYIEEISCASDQVEKYKLDIAQLEENLKEKNDEILSLKQSLEENIVILSKQVEDLNVKCQLLEKE
              |||+|||||||| ||||||||||||+|+||||||| |||| ||||||||||||| + ||| ||| ||||||| |
Mouse:   63 DEKCETEKLLEYIQEISCASDQVEKCKVDIAQLEEDLKEKDREILSLKQSLEENITF-SKQIEDLTVKCQLLETE

Human:  301 KEDHVNRNREHNENLNAEMQNLKQKFILEQQEHEKLQQKELQIDSLLQQEKELSSSLHQKLCSFQEEMVKEKNLF
              ++ |++ ||+ |+|+|||| | ++++||+|| ||||||||| |||||||||||+ | |+|||||||| |||+|
Mouse:  138 RNDLVSKDRERAETLSAEMQILTERLALERQEYEKLQQKELQSQSLLQQEKELSARLQQQLCSFQEEMTSEKNVF

Human:  376 EEELKQTLDELDKLQQKEEQAERLVKQLEEEAKSRAEELKLLEEKLKGKEAELEKSSAAHTQATLLL--------
              |||| | ||| +||||||+||||||||||| || ||+| |+ |+ || |||| ||| || |+
Mouse:  213 KEELKLALAELDAVQQKEEQSERLVKQLEEERKSTAEQLTRLDNLLREKEVELEKHIAAHAQAILIAQEKYNDTA

Human:      --------------------------------------------------------------------------

Mouse:  288 QSLRDVTAQLESVQEKYNDTAQSLRDVTAQLESEQEKYNDTAQSLRDVTAQLESEQEKYNDTAQSLRDVTAQLES

Human:  443 QEKYDSMVQSLEDVTAQFESYKALTASEIEDLKLE*NSSL*QEKAAKAGKNAEDVQHQILATESSNQEYVRMLLDLQ
              ||||+ ||| |||||| ||||+ | |||||||||| +|||| | | |+ |||| ||| ||+|||| ||+ |||
Mouse:  363 QEKYNDTAQSLRDVTAQLESYKSSTLKEIEDLKLENLTLQEKVAMAEKSVEDVQQQILTAESTNQEYARMVQDLQ

Human:  518 TKSALKETEIKEITVSFLQKITDLQNQLKQQEEDFRKQLEDEEGRKAEKE*NTT*AELTEEINKWRLLYEELY*NKTK*
              +| ||| ||||||| |||+|||||+|||+||+||||||||++ | ||||| ||| |||||||||||||||| |||
Mouse:  438 NRSTLKEEEIKEITSSFLEKITDLKNQLRQQDEDFRKQLEEKGKRTAEKENVMTELTMEINKWRLLYEELYEKTK

Human:  593 PFQLQLDAFEVEKQALLNEHGAAQEQLNKIRDSYAKLLGHQNL**KQKIKHVVKLKDENSQLKSEVSKLRCQLAKKK**
              ||| ||||||| |||||||||||| |||||||||||||||+||||||||||||||||||||||||||||||+|| |+|
Mouse:  513 PFQQQLDAFEAEKQALLNEHGATQEQLNKIRDSYAQLLGHQNL**KQKIKHVVKLKDENSQLKSEVSKLRSQLVKRK**

Human:  668 QSETKLQEELNKVLGIKHFDPSKAFHHESKENFALKTPLKEGNTNCYRAPMECQESWK*
              |+|++|| ||+| |||+|||||||||| |||||| | |||||   ||||||||| ||
Mouse:  588 QNELRLQGELDKALGIRHFDPSKAFCHASKENF---TPLKEGNPNCC*
```
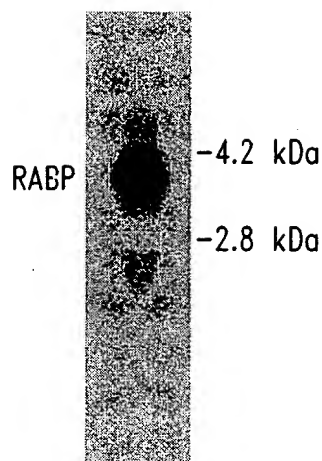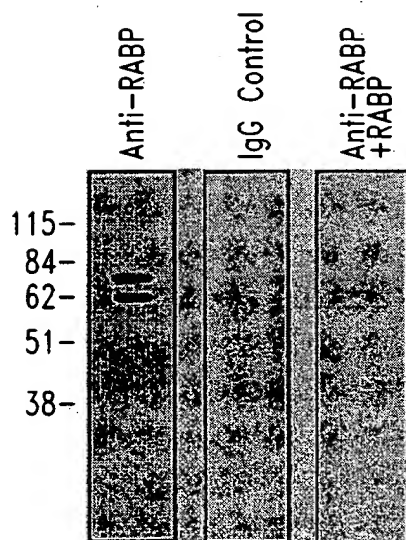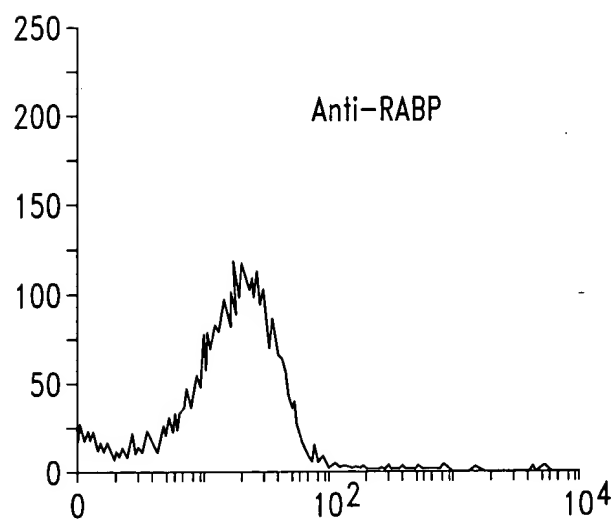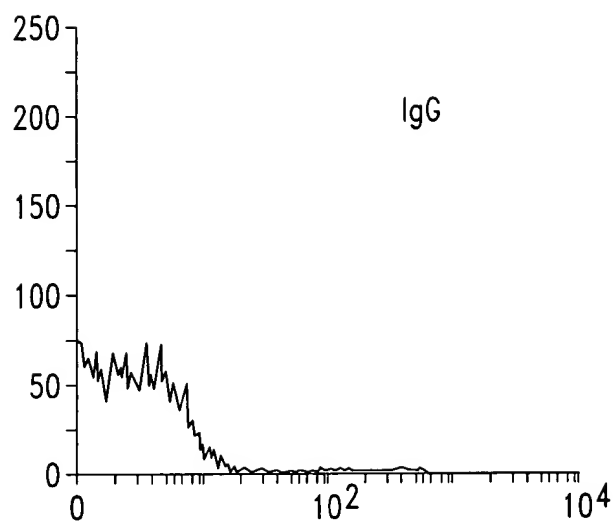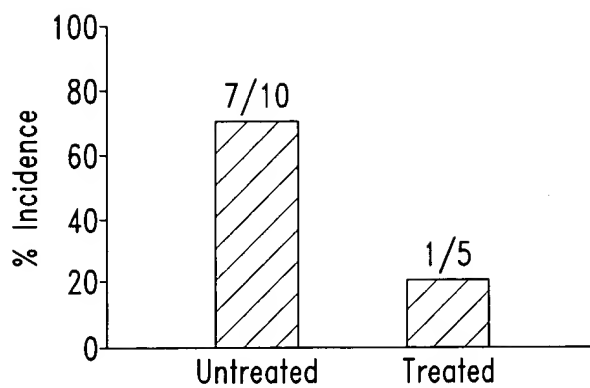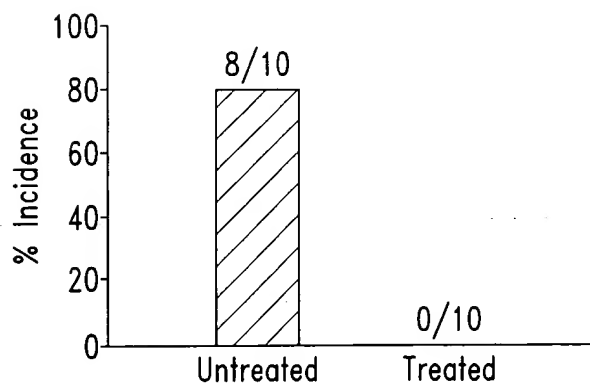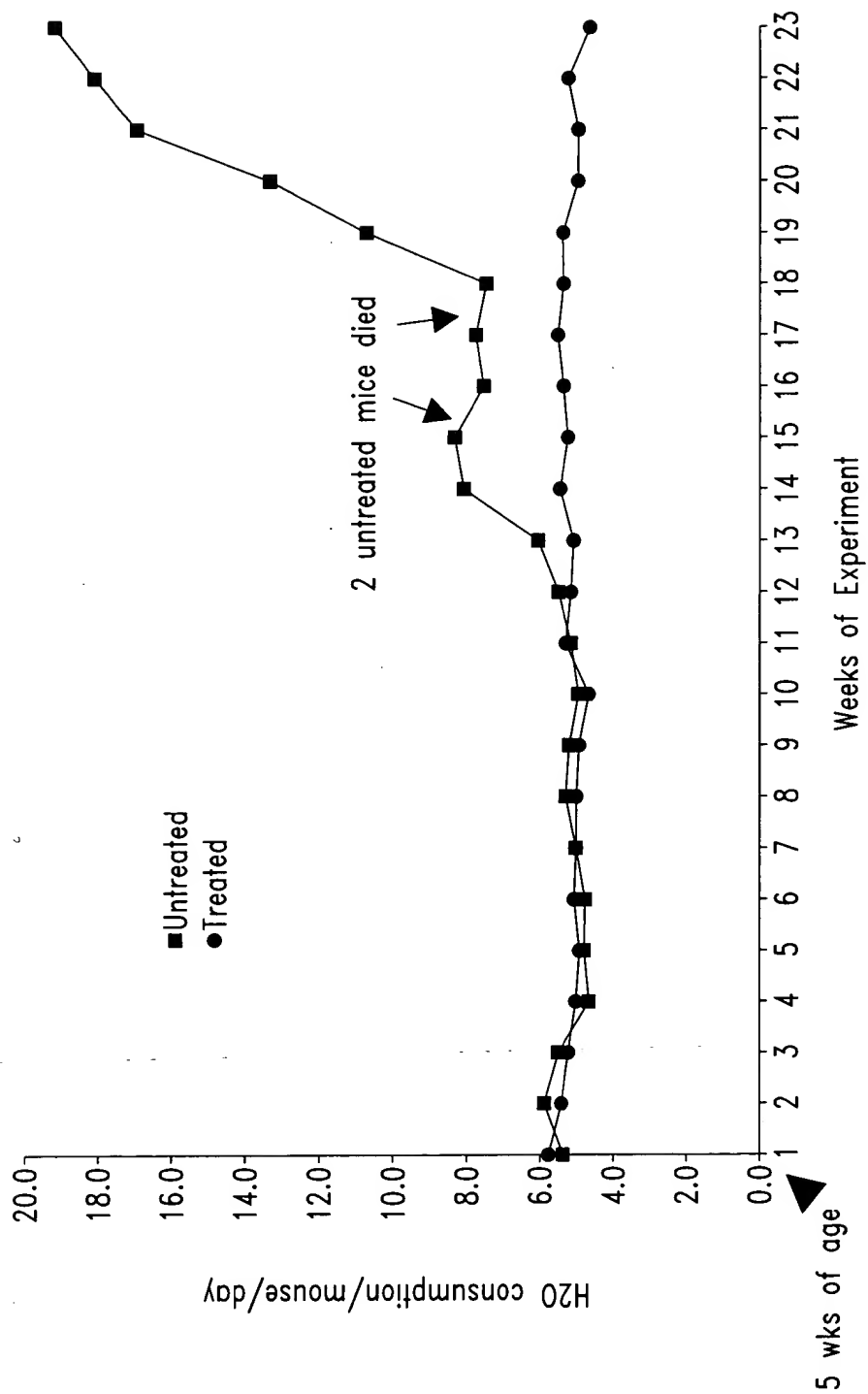
Fig. 50

Note: normal blood glucose level = 99-140
Incidence of abnormal blood glucose level in NOD mice

Fig. 51
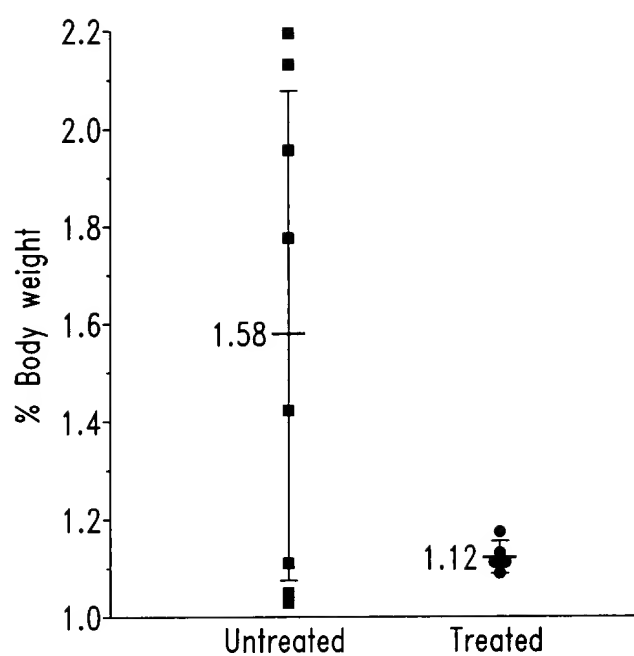

Incidence of abnormal urine glucose level in NOD mice

Fig. 52

Effect of P-16 peptide on water consumption in NOD mice

Note: Increased H2O consumption indicative of Diabetes Insipidus, a complication of Diabetes Mellitus

Fig. 53

Note: 2 untreated animals died during the course of experiment
Effect of P- 16 peptide on kidney weight in NOD mice

Fig. 54